1.

The main goals of this project are the application of Lab-on-a-chip technology to the detection of the aquaculture original illness-causing bacteria; the integration among aquaculture biological technology, microchannel system technology and fluorescence detection system technology; the development of portable micro detection system for aquaculture original illness-causing bacteria; the placement of aquaculture pond water detection specimen onto the microchannel chip system for the executions of three steps such as, the rapid extraction of RNA, simple purification and precise mixing; the use of SSU RNA oligonucleotide unique section as probe, meanwhile, mark it with fluorescence and mix it in the preset environmental conditions, measure the fluorescence signal through optoelectronic system in order to identify aqua original illness-causing bacteria and judge the existence of aqua original illness-causing bacteria, the result is to be used as a reference for whether the antibiotics should be added to the fish food; therefore, this project proposes the concept of the application of unique probe, which combines the microchannel technology, to the detection of specimen in the aquaculture site, it takes only the drop of the pond water specimen onto a chip of the size of a coin, or the touch of cotton swab on the infected dead body floating on the top of the pond water and put them onto the chip, the result can be obtained in short time and used as a reference for pond management, the greatest advantage is to control the cost of unnecessary feed of antibiotics from the aquaculture pond owners, it also reduces the consumption of antibiotics which in turn enhances the safety of aquaculture product.

2.

The overall project and sub-project 3 leader, professor Chung-Cheng Chang, has his research interest for the recent 5 years focused on the integration of MEMS, optoelectronic, preparation of integrated sensor device using IC manufacturing technology, he integrates the short wavelength optical sensor devices he has completed in the past such as: ZnSe/Si schottky photodiode, ZnSe/Si PIN photodiode, ZnSe/Si HBT, n-ZnSe/p-Si/n-Si Heterojunction phototransistors, ZnSe/Si MSM photodiode, n-ZnSe/p-GaAs/n-GaAs HBT, in the initial stage, the preparation of a single chip integrated from ZnSe/GaAs and ZnSe/Si optical sensor device and gain-enhanced device has been completed, in the same time, the related optoelectronic characteristics test system has been set up. Besides, the integration of IR sensor device, pressure sensor, ultrasonic device and amplifier into a single chip has been completed, the research result has been applied recently, these include IR array device, ultrasonic array device and biological and medical device, etc.; the development of biological and medical chip is to integrate MEMS and optoelectronic technology for the integration of microchannel based on polymer material and the fluorescence detection system. The fluorescence detection system has been built up in the initial stage, the real specimen measurement has been done with the help from professor Chao-Te Chen. If the home-made Sensor and micro flow system can be integrated, the Lab-on-a-chip work platform can then be completed.

The sub-project 1 co-leader of the overall project is professor Chao-Te Chen, his expertise are: (1) Biology, biochemical and molecular biology, (2) fishery and ocean (3) biological technology (4) microbe, his research interest includes: transgenic gene, biological technology, molecular biology.

The infection of fishes in the aquaculture ponds by original illness-causing bacteria usually causes serious loss to the aquaculture pond owner, therefore, the government has spent lots of efforts in the study regarding the prevention of fish diseases.A. hydrophila and E. tarda are the commonly seen aqua original illness-causing bacteria in the non-salt water aquaculture pond. It has taken a project sponsored by National Science Council in the initial setup of this lab, the project aims at studying the illness-causing mechanisms of the aqua original illness-causing bacteria, it is the first lab to complete the blood-dissolving gene cloning and sequencing of E. tarda, it also analyzes the biochemical characteristics of hemolysin. From the study results understanding and analysis, we propose different fish disease prevention concept, that is, to control the infection from the original illness-causing disease through the improvement of aquaculture pond environment, this is to replace the conventional medical way for a doctor to treat human body or for an animal doctor to treat the individual animal, the former way of improving the pond environment is more feasible. Besides, we also take the applied project from Council of Agriculture, Executive Yuan, to study the early diagnosis and develope the technology to foresee the infection from original illness-causing bacteria. In 2001, the research is stopped for 2 years due to bad health condition, the research work ahs been recovered since last year, A. hydrophila and E. tarda unique probe design and test feasibility study have been completed, the result ensures that AH642 and ET996 probe can precisely detect the commonly seen bacteria A. hydrophila and E. tarda. This year, an unique oligonucleotideprobe is used to mix and detect the preserved bacteria frozen at -80℃ for one year, eight illness-causing genes of Bacteria of Edwardsiella tarda such as: hlyA, citC, fimA, gadB, katB, mukF, orfA and ssrB are detected respectively to identify the relationship between RNA performance and illness originality. When Tilapia is used for abdominal injection attack test for the original illness-causing bacteria, the dead fish can be re-separated the bacteria from the liver, ET996 hybridization and identification is used and verify the bacteria to beBacteria of Edwardsiella tarda. Meanwhile, RNA is extracted and unique probe mixing and inspection has been done, the result shows that the RNA of the eight illness-causing genes will all perform, but the live fish seven days after the attack test can not be separated for the bacteria after blood specimen is taken from the live fish, it shows that whether the RNA of the eight illness-causing genes performs or not indeed is related to the illness caused by the infection from the original illness-causing bacteria.

The co-leader of the sub-project 2 of the overall project, professor Po-Tai Chen, his expertise includes: (1) Structural acoustics (2) mechanical engineering, (3) MEMs (4) Electrical mechanical integration, his research directions include: microchannel, ultrasonic array.

The applicant has micro technology related researches such as: the preparation of micro capacitor type ultrasonic device and the investigation of microchannel related technologies in the recent two to three years, among them, the micro capacitor type ultrasonic device has been tested to be consistent with the theoretical model. The current research direction is toward the preparation of array ultrasonic device, this project is one of the sub-project of a three year integration project from the National Science Council, its goal is to use two vertical one dimensional array devices to generate a two dimensional array transport and reception system, it can be applied to the applications such as: underwater image formation and biological and medical image formation.

Many different technologies have been applied in the preparation of microchannels, these include sacrificial layer technology, SU-8 and PDMS, etc., here the fluid manipulation technology is emphasized, and the heated air is used as the driving force to drive the fluid, meanwhile, PID and gradual approximation method are used for the temperature control. The resistance type temperature sensor is used for temperature measurement, only one resistance device needs to be calibrated among many different resistance sensors in the same chip, the result is independent of the resistance device dimension. These results are published in the domestic meeting, paper or thesis is written from them for both domestic and oversea journal.

Besides, a study two to three years ago by the applicant has focused on the interaction between the structure and the fluid acoustics as well as structure dynamics, a software has been developed to calculate the acoustic radiation problem for submarine and floating body under the water, it can be used in the design and evaluation of military ships, currently, the software has been transferred to the domestic United Ship Design and Development Center.

The co-leader of sub-project 2 of this overall project, assistant professor Chih-Wei Wu, his expertise include: (1) MEMs (2) semiconductor processes and equipments, (3) formation technology of composite material, (4) planning, installing and operation managing of MEMs system R&D alb.

1.1 Surface acoustic wave filter development for wireless communication: The surface acoustic wave device has the advantages such as: high performance, small size, low cost and compatible to the IC process technology, therefore, it plays an important role in the electronic industry and biological and medical industry. Later on, I join the research group lead by professor Cheng-Chung Wu of Institute of Applied Mechanics in National Taiwan University, an surface acoustic wave device with a central frequency of 400 MHz has been prepared on the quartz piezoelectric substrate by using MEMs technology, this study result has produced a paper published overseas（SCI）and a paper published domestically in a seminar.

1.2 The development of Diffraction type laser optical meter system: Highly sensitive and precise measurement is indispensable technology for nano and micro research work. This study has successfully produced a new diffraction type diffraction type laser optical meter system, it also produces a diffraction type device board receiver, this study has produced successfully an oversea seminar paper and two domestic seminar paper.

1.3Electronic packaging study for micro sensor device: This study has designed a MEMs packaging process which can be integrated with semiconductor technology, the design features include: stereo structure packaging capability, hermetic environment and high adhesion strength, high process compatibility, a cost reduction due to array type manufacturing, low temperature packaging, etc.The study result has successfully produces two domestic seminar paper.

1.4 Development of Pyroelectric infrared sensor technology: This study uses MEMs process to miniaturize the sensor in order to prepare pyroelectric film infrared sensor, it also studies the sensor’s response to infrared frequency. It is verified that pyroelectric sensor can sense near infrared and the far infrared range of human body, this characteristic make it suitable for human body temperature measurement, the result has produced successfully a domestic seminar paper.

1.5 The study of the application of new type highly sensitive micro suspension arm biological sensor to the immune analysis method: This study uses MEMs technology to prepare a micro suspension arm detector chip to perform protein immune analysis, meanwhile, electric field is used to separate the antigen which is combined with antibody, good reproducibility is maintained so that rapid and precise result can be achieved, the result has lead to a successful oversea seminar paper.

The co-leader of sub-project 4 of the overall project, assistant professor Chih-Chieh Ho, his expertise include: (1) Micro Optical Electro-Mechanical technology and engineering, (2) Sensor circuit engineering, (3) Optoelectronic engineering (4) Semiconductor device design, his research interests include: optoelectronic device and the application of Micro Optical Electro-Mechanical system technology in the preparation of sensors.

Until the end of 2004, Dr. Ho has his main research results published in famous journals and seminars such as:IEEE, JECS, IEE EL, JEM, SSE, etc. (For the detailed list, please refer to the publication item in the bibliography), a total of: 50 paper in the journal (International 39, domestic 11), most of the international journals are good periodical of SCI; 50 paper for the seminars(International:31, domestic: 19). His overall research in optoelectronic category include: display device application technology, low temperature growth of optoelectronic device using Metallic Longitudinal Induced Crystallization (MLIC) technology, high performance far infrared optical sensor and the preparation of integrated sensor device which is integrated from PIN optoelectronic device and Avalanche Photodiode (APD) which absorbs avalanche separation.For the sensor preparation based on Micro Optical Electro-Mechanical System technology include: the preparation of optical micro sensor, chemical micro sensor and mechanical micro sensor through MEMs technology.

The co-leader of sub-project 4 of the overall project, associate professor Wan-Jung Liu, his interests include (1) Analog integrated circuit design, (2) Mixed mode integrated circuit design, (3) High frequency integrated circuit design, his research direction include: ASIC, application specific integrated circuit design, design and application of optoelectronic system, IC process simulation, radio frequency integrated circuit design.

In the recent 5 years, his research mainly targeting at the study of analog signal processing and mixed mode signal processing, he has developed the interface integrated circuit for analog and digital circuit with different functions, for instance, he has developed Delta- Sigma analog digital converter (ADC) with high resolution MASH architecture which is used in the voice signal processing and Delta-Sigma digital analog converter (DAC), such ADC/DAC has been commonly used in the voice application. Besides, in addition to the successful development of ASIC which combines color conversion processing and can be applied in the scanner image processing, he has devoted to the development of high speed ADC to be applied to the image processing, this ADC is based on parallel pipelined architecture, with 10 bit resolution, its sampling frequency can be greater than 150Mbps, this ADC can be used in high speed image processing, for instance, large size (above 17 inches) liquid crystal display and high resolution TV, this ADC is one of the most wanted IP for domestic IC design house.

He has devoted also to the design and development of radio frequency (RF) integrated circuit, such

high frequency integrated circuit has its main application in the circuit development of receiver and emitter of high frequency wireless communication, besides, it is also applied in the optical communication transceiver circuit with frequency above OC-48. The developed RF integrated circuit include: 2.4GHz receiver, power amplifier (PA) of 2.4GHz emitter, low noise amplifier of 5GHz, 5GHz integral type phase locked loop (PLL), etc., the current project combines the past developed PLL technology and the Delta-Sigma ADC modulator circuit technology in order to develop a high quality, low noise, high resolution, adjustable, 5GHz non-integral type frequency synthesizer, such frequency synthesizer is very complicated and challenging, it is more difficult especially when it requires low power consumption design, this frequency synthesizer is going to be used in the circuit development of 5 GHz high performance receiver.

In order to be used in the optical fiber communication and the front end receiver circuit of 10 G bit high speed Ethernet, Transfer Impedance Amplifier (TIA) and Automatic Gain Control(AGC) circuit are some of the key technologies. Since the Transfer Impedance Amplifier (TIA) needs functions such as: high bandwidth, high transfer impedance gain, low voltage, low power consumption and low noise, and the Automatic Gain Control circuit needs to cover very broad input range and enough bandwidth in order to meet the requirements of bit error rate and random noise. Therefore, the development of Transfer Impedance Amplifier (TIA) and Automatic Gain Control (AGC) is highly challenging due to its high speed and low noise requirements, it is more difficult than the front end receiver circuit of common Ethernet and more challenging.

3.

The main purpose of this project is to integrate aquaculture biotechnology, microchannel system technology, and fluorescence detection system technology so that a portable aquaculture original illness-causing bacteria micro detection system can be developed. In addition, the system will be a carrier to fulfill the goal that each aquaculture unit can detect the infected bacteria from the infected sick fish independently, furthermore, the detection of whether the aqua original illness-causing bacteria exist or not in the aquaculture pond can be used as a reference for the pond management, therefore, the final goal of the reduced usage of antibiotics in the aquaculture can be achieved, and the aquaculture food safety can be guaranteed, the goal of reduced antibiotics usage cost can be realized. The developed simple lab chip can be further used as a rapid quarantine tool for the import of live aquaculture species at the Customs.

The advanced and well developed countries around the world nowadays keep finding a new high growth rate technology for the next century industry. And the broad definition and wide application scope of biotechnology meets the goal pursued in this direction of thought, therefore, the advanced countries head rapidly toward the development of biotechnology through interdisciplinary integration of newest sciences, biology, medical science and engineering technology, they all view it as an important technology to maintain the overall national industry growth. The booming in biotechnology, its rapid development step and rapidly expanded application scope has shifted the research paradigm in the biosensor to a main axis based on multifunction, miniaturized and parallel processing chip concept. Extending from this, the concept of「system in a chip」has become the mainstream in the current research

and development activities in the biotechnology and medical science fields. The application of semiconductor manufacturing technology originated from 1990 has brought brand new technology to the development of this field, the microlithography technology and mass production has brought the improvements on the micro analysis, high speed detection and cost performance, the technologies in Micro Electro-Mechanical System (MEMs) has further brought unlimited space for the design and manufacturing of "system on a chip", the characteristics such as high degree parallelism, automation, high mass production, micro volume, and high speed, etc. are going to be the continuous development key points in the semiconductor and MEMs technologies.Therefore, if the originally complicated and tedious lab work procedures and setup can be concentrated on a lab chip system through the design and manufacturing of planar nano and micro device (Lab on a chip, LOC), the whole detection is going to be very rapid and simple, in addition, the biological detection efficiency can be enhanced and the application scope can be broadened, this has become one of the newest and important research paradigm. With this technology, the conventional process flows and requirements can be neglected, for instance, the need of enough samples accompanied with the separation technologies before the qualitative or quantitative examinations or characteristics identifications can be performed, the conventional way not only takes a long time but needs to be performed in the lab. Recently, many research units in the research institutes and companies has paid great attention to the potential of LOC, they have started to perform researches in related fields, the biochip research group in this university experiences of many years in this field, the study of this project aims at solving the problems of time-consuming and can't be processed on site for the original illness-causing bacteria detection in the aquaculture business through the use of LOC technology.

The massive death of fishes in the aquaculture pond has happened once a while, from the study results of past few years, we know that it takes about 2 to 4 weeks from the infection of pond water or the ability to separate original illness-causing bacteria from some infected sick fishes to overwhelming infection, therefore, it is desired to examine *A. hydrophila* and *E. tarda* for a pre-sorting before the explosion of massive infection. There is no need to feed antibiotics if there is no finding of original illness-causing bacteria, it is more appropriate to treat whenever the existence of original illness-causing bacteria is detected and there is the possibility of the explosion of massive infection, this way it could reduce the remaining trouble of antibiotics, but the prerequisite must be the detection of original illness-causing bacteria before the explosion of massive infection. Conventionally, agar is used for the growth of colony before many following biological detections are done, the whole process could take at least two to three weeks, and if the fishes in the pond are infected, the loss must be serious, there is not enough time at all to do appropriate treatments, therefore, most aquaculture owners feed the pond and fishes with antibiotics regularly for infection prevention. The routine feed of antibiotics not only cause the anti-medicine property of the original illness-causing bacteria but also cause food safety problem due to antibiotics remaining, meanwhile, there will be nearly no chance once the fishes are infected, therefore, it is always an important topic in the fish disease prevention area to develop rapid detection technology for the detection of original illness-causing bacteria.The concept of Lab-on-a-chip has stepped into practical stage due to the rapid development in microchannel technology, use the unique oligonucleotide probe of SSU rRNA to mix and inspect the original illness-causing bacteria and

illness-causing gene, it becomes more feasible to be applied to the forecast of the timing of the occurrence of fish illness if the detection time can be reduced to within one hour, the operation is easy and the price is low. Therefore, our research group propose a concept to use the unique probe based on LOC technology in the aquaculture pond for the specimen detection, it only needs to drop the pond water specimen onto a chip of the size of a coin, or one can uses a cotton swab to touch the dead infected fish floating on the water surface of the pond to take specimen onto the chip, the test result can be obtained in short time and used as a reference for management, the greatest advantage of this is to control the unnecessary feed of antibiotics from aquaculture pond owners, this not only saves the medicine cost but guarantees food safety, in addition, it is a good tool to be applied to the live aquaculture species quarantine. Currently, professors from the electrical engineering department, system engineering and naval architecture department and mechanical engineering department in this university are developing LOC technology, therefore, we can integrate aquaculture biotechnology, microchannel system technology and fluorescence detection system technology in this university in order to complete the development of a portable micro detection system for aquaculture original illness-causing bacteria, meanwhile, use the unique 16S rRNA oligonucleotide section developed by the aquaculture biotechnology lab in this university, we can develop together a set of technology for the rapid detection of the aqua original illness-causing bacteria in the aquaculture pond, use this technology in the forecast and sorting of the occurrence of the original illness-causing bacteria and the result can be used as a reference of whether antibiotics should be fed to the pond, moreover, we can further develop a chip for the detection of commonly seen or legally defined original illness-causing bacteria in the aquaculture species or use it as the best tool for quarantine of aquaculture products.

According to the statistical data published by the Council of Agriculture in 2003, the total aquaculture pond area in Taiwan is about 28790.95 hectares. If we do an estimation purely based on the economic production value of the chip itself, suppose 5 ponds are contained in the area of one hectare and one detection is performed on each pond every two weeks by using the chip, also suppose the cost of using each chip is 50 New Taiwan dollars, then the annual production value in a rough estimation is about 187 millions New Taiwan dollars. In addition, according to the imported animal antibiotics data published by the Ministry of Economic Affairs for the year from 2001 to 2003, it's a total of about 2.03 billions New Taiwan dollars, suppose the aquaculture business consumes 1/3 of the total, just the C.I.F. price each year will consume 0.225 billion New Taiwan dollars from the importers, therefore, we estimate the actual antibiotics amount consumed by the aquaculture pond owners should exceed 1 billion New Taiwan dollars /year. Suppose the detection of original illness-causing bacteria and the probability of the necessity to use antibiotics is reduced to 1/10 each year, the total cost saved is thus about 0.89 billion New Taiwan dollars/ year.

The chip system detection not only can reduce the cost of unnecessary use of antibiotics in the aquaculture business, the most important thing is to protect consumer's health. In addition, the chip itself represents a product of advanced technology, even in the aquaculture advanced countries such as Japan and Norway, they still can't achieve the forecast and sorting technology for the original illness-causing bacteria, therefore, the idea of the development of this detection chip system possesses innovative and forethought characteristic.The development of original illness-causing bacteria detection

system for the aquaculture pond in this university is an integration of the existed research results from Dr. Chao-Te Chen from the Department of Aquaculture in the College of Life and Resource Sciences and the LOC chip technology currently under development by the engineering related departments in this university, it possesses the characteristics of innovation, forethought and importance both academically and technically, in addition, it could also enhance the aquaculture product quality for the domestic owner of this business and reduce the danger from the remaining of antibiotics which in turn increases the product's international competitiveness. In the academy and industry cooperation aspect, the domestic DR. Chip Biotech Inc. has the willing to cooperate with the biochip research group in this university, the system after integration can be possibly developed to be mass producible product so that the product application scope of the domestic biochip company can be broadened, the manufactured LOC chip not only has great market potential in Taiwan, but in Mainland China as well as Southeast Asia. The execution of this project can also let the domestic biochip manufacturers step into the detection system for aquaculture business earlier so that their international competitiveness can be enhanced.

4.

The main goal of this project is to integrate and prepare a portable Lab-on-a-chip micro detection system for aquaculture original illness-causing bacteria so that the aquaculture pond owners can detect themselves whether the original illness-causing bacteria in the water of the aquaculture pond meets antibiotics feed requirements and thus antibiotics feed can be properly controlled and the ponds can be well managed. In addition to the saving of antibiotics cost, it can also reduce the danger of antibiotics remaining due to improper feed of antibiotics by the aquaculture pond owner, and the safety and health of aquaculture fish product can be ensured.

This detection chip is an integrated system, it includes technologies such as: specimen processing flow, injection system, preparation of microchannel system, microchannel fluid dynamic design and measurement analysis, specimen collection, detection, analysis and display, etc., without any single one, a complete and rapid micro detection system can not be built. Therefore, there are four sub-projects in the overall project: the integration relationship of each sub-project is as shown in figure 1, and the specimen detection system flow is as shown in figure 2.

In the initial implementation of the overall project, all the sub-projects will be integrated and coordinated in order to start some related works according to the schedule and finally the overall project schedule can be well followed. Meanwhile, the common development items in the related sub-projects will be coordinated in order to enhance the overall R&D efficiency, the overall project will select the meeting discussion time of each group to well grasp the project schedule. The overall project will perform the preparation of biological specimen together with the sub-project 1: the biological detection specimen will be obtained from the Aquaculture Department and Biology Department in this university. For example: protein, DNA, RNA, fluorescence dye, or even virus. Fluorescence detection system will be built up according to the requirement in sub-project 1 and together with sub-project 3. Then the built fluorescence detection system will be experimentally verified for its capability to be applied to the detection of original illness-causing bacteria with the help from sub-project 1 and 3.

Besides, we will help sub-project 2 to prepare microchannels: a layer of SU-8 photo resist will be spin-coated onto the silicon substrate, exposure action will be performed accompanied with the prepared photo mask. After developing, IPA will be used to rinse to complete the preparation of mother mold. Furthermore, pour the prepared PDMS onto the mother mold and bake it in 120 ℃ for one hour. The solidified PDMS structure is then separated from the mother mold. Then use O_2 plasma to treat and combine the PDMS and glass substrate to complete the preparation of microchannels, this method has the advantage of low manufacturing cost and suitability for mass production. In addition, sub-project 1 and 3, co-leader professor Sheng-Yu Chao, Dr. Chih-Yen Cheng, will be coordinated to perform detailed design and test on microchannels so that it can be optimized. In addition, sub-project 3 and 4 will be coordinated to perform the design and test of of fluorescence detection system and sensor. Moreover, sub-project 2, 3, 4 and Dr. Chih-Yen Cheng will be coordinated to perform the detailed design and test of Hybridization chamber. In addition, sub-project 1, 2 and 3 will be integrated in order to perform the test in the Hybridization fluorescence detection process. In the gene point array technology, Probe, usually different kinds of DNA or cDNA are fixed on the surface of the carriers which have been chemically surface-coated and treated by point array, and the target to be tested and Probe are then hybridized. Since DNA is of double helix structure, it possesses the complementary and unique property, this is like the property of a zipper, the target nucleic acid in the target to be tested will be hybridized and fixed on the Probe points of the complementary nucleic acid series contained in the cDNA micro point array glass; then rinse it to remove the sample nucleic acid which has not been hybridized and to record the locations of points which involve hybridization reactions. Let the laser light from the self-installed green laser in the lab pass through a tiny optical fiber to obtain sufficient self phase modulation effect, a very broadband optical source will then be generated, then inset the optical fiber into the self-made PDMS microchannel biochip. The green laser beam will pass a beam splitter and be separated into reference optical path and specimen optical path. The specimen will be placed on a stand and processed by DAQ card of Labview, then the result will be displayed by a LCD display.

In addition, sub-project 4 and Dr. Wei-Chung Ting in Opto-Electronics and Systems Laboratories of ITRI will be coordinated to perform the test and analysis of thin film deposition, and an optical sensor of Poly-$Si_{1-x}Ge_x$ containing a filter will be further prepared. Meanwhile, the overall project will be coordinated with sub-project 1, 3 and professor Jung-Hua Wang to process the obtained signal and the later test data will be handled and analyzed, meanwhile, sub-project 3, 4 and professor Jung-Hua Wang will be coordinated to process the obtained signal and display the measured value on the LCD screen by using the designed and prepared IC. System integration and test will be performed after initial result has come out from the coordination and integration of all the sub-projects.

The mutual integration of all the sub-projects: After the biological specimen of sub-project 1 is prepared by the Aquaculture Department in this university, it is then injected the specimen input area of the microchannel prepared by sub-project 2 and is driven by the micro pump. Then it will enter the filter to filter out the unnecessary specimen and then pass through a second micro pump to have a RT-PCR treatment with the buffer solution. Then it will pass a third micro pump and get hybridized as in sub-project 3 and detected by the SiGe sensor in sub-project 4. Finally, the Labview platform

installed by sub-project 5 will be used as system operational interface and signal processor.

5-A.

The main purpose of sub-project 1 is to improve the complicated lab big specimen sampling process flows and turn it to micro size, to design the reaction process accompanied with microchannels and to enhance the detection sensitivity and precision. The experimental method is based on the unique probe of A. hydrophila and E. tarda, it compares and analyzes the conventional detection results in the lab and the signals passed through proto type microchannel chip. The simple biological sample processing flows by the chip microchannel can be divided into three steps, these include the cell dissolution and the release of RNA, fast pass through the chromatography column and the purification of RNA, and the precise mixing of the RNA by the fluorescence-marked probe after it adsorbs to the Nylon film.The sub-project 1 will provide standard experimental flows for the lab, meanwhile, it will simulate the feasibility of the micro-content reaction occurred in the chip microchannels, each reaction process flow will be improved accompanied with the microchannel design in the sub-project 2. The fluorescence-marked probe used will be accompanied with the optoelectronic detection system in sub-project 4, fluorescence which can be used to mark oligonucleotide will be carefully selected so that the signal detection sensitivity and precision can be greatly enhanced. In addition, a unique probe for important aquaculture fish commonly seen original illness-causing bacteria will be developed in order to increase the practicality of advanced and improved microchannel chip.

5-B.

The goal of sub-project 2 is to propose a complete design method for microchannel, which includes functions such as quantitative analysis, driving, mixing and temperature reaction of microchannels, these are to build a structure of microchannel system for the Lab-on-a-chip. In this project, microchannels are going to be prepared in the biochip, and the processing steps such as biological molecule replication and specimen transport will be completed by the operation of fluid system.In order to achieve the goal of high productivity and reduction of manufacturing cost, this project is going to obtain microchannels and microchambers of fixed volume on the glass substrate and plastic polymer material (PDMS) through technologies such as micro lithography, etching, film deposition, thermal pressing molding, etc., and finally, UV adhesive will be used for low temperature packaging and combining of the two parts. For the pre-treatment of the specimen, slot channels for the installation of filtering film are installed on the front end of the micro fluid system, the filtering film has hole diameters of 200μm, 5μm, 0.2μm, respectively, it can sufficiently separate and purify the target to be detected in the specimen. Besides, temperature-sensitive hydrogel is further used for the preparation of microvalve, it is used for the process adjustment and control so that the control of fluid flow can be achieved.In the control part of the fluid system, biological molecule surface dynamics will be investigated, multiplexing precise control of liquid transport for medium scale fluid channel in the chip will be performed through a design of externally added pump or electric field, this is to facilitate the parameter study of biological molecule reaction mechanism. The driving force for this project will be mainly from the pneumatic

or liquid pressure pump driven by piezoelectric crystal, its advantage is to produce enough force and fast response time (1msec). In addition, small quantity specimen and reagent means fast response and high sensitivity, the control of micro fluid in the reactor for this sub-project, which includes mixing, rinsing, shaking, etc., will be handled as water drop, figure 3 shows water drop controlled by surface tension.

The study of the design, manufacturing and application of biological detection chip all involve the processes of mixing, rinse and separation of biological specimen and the target. In the micro fluid field, since the fluid field dimension is small and fluid speed is slow, the movement of the fluid is mainly affected by the viscous property of the fluid and the surface tension of the interface between the fluid and the solid microchannels. These two interactive forces are not advantageous for the mixing between the biological specimen and the target. How to enhance the effects of mixing and separation of different media in the micro fluid field is one of the main topics of this project. In order to enhance the mixing effect in the fluid field, a better way is to let the fluid mass point in the fluid field jump, and this goal can be achieved by using the small jet generated during the breakage of bubble in the fluid field. In the sub-project, experiment will be performed in the flow channel of centimeter size, mass point image velocity measurement method (PIV) will be used measure the enhancement effect of the mixing effect from the bubble breakage fluid field on the low Reynold number fluid field. Then bubbles will be generated and broken in the prepared microchannels, and use microscope to measure the mixing, rinsing and separating effects in the micro fluid field by using Micro-PIV method, the result is to be used as a reference for the modification of the preparation of microchannels, finally, microchannel system design of biological detection chip can be optimized.

5-C.

The main goal of sub-project 3 is to complete the build up of hybridization and detection chamber and the system integration, some key jobs in this project are to perform hybridization process flows and system build up in accordance with sub-project 1 and sub-project 2 and perform the interface preparation integration between microchannel and fluorescence detection system in accordance with sub-project 2 and 4. The working items in this project are first to allocate different DNA reception bodies onto the Nylon film in accordance with sub-project 1 and sub-project 2, meanwhile, a design integration on the Nylon size and the hybridization and detection chamber size will be performed in accordance with sub-project 2. In addition, fluorescence detection system including laser and photo detector will be built up so that independent external test can be performed, test sample will be provided by sub-project 1 for fluorescence detection, then, standard Probe preparation and placement flows will be built up in accordance with the hybridization and detection chamber of sub-project 2, 4. Meanwhile, build fluorescence detection system on the Chamber, complete the combination between optical detection system chip, which integrates Sensor and single chip, and microchannel system chip so that a single LOC chip can be formed and the whole system becomes one step closer to perfect, a biochip detection system which includes the integration of microchannel system and a fluorescence detection system, which further includes sensor devices, laser, single chip, is then built. Meanwhile, the specimen planned

by sub-project 1 will be used for integration test in accordance with the overall plan, finally, an integrated portable micro detection system for aquaculture original illness-causing bacteria detection is thus prepared.

Sub-project 4 will be based on the past research results, Metal-Induced Longitudinal Crystallization (MILC) technology will be used to grow Avalanche Photo Diode(APD)device based on poly-silicon germanium (**Poly-$Si_{1-x}Ge_x$**)thin film at low temperature；The growth of **Poly-$Si_{1-x}Ge_x$** thin film based on MILC technology can effectively prevent the change of carrier transport property due to metallic contamination, meanwhile, there is still no literature published about its application to color filtering APD sensor device, therefore, it is an innovation research direction. The experimental method used in this study is to develop key technologies related to the growth of **Poly-$Si_{1-x}Ge_x$** thin film based on MILC technology at low temperature as well as characteristics analysis, for instance, the growth temperatures, the effect of silicon and germanium composition on the growth temperature, resistance analysis, **Poly-$Si_{1-x}Ge_x$**/Si epitaxial interface characteristic and technologies such as horizontal crystallization rate, etc.; meanwhile, combine the porous silicon surface nano structure technology to develop and improve the quality of APD photo sensor based on **Poly-$Si_{1-x}Ge_x$** thin film material, finally, apply such detector in the fluorescence detection system. Moreover, an APD sensor device which possesses the color filtering function will be developed by using the above-mentioned research results, its main structure include: micro biochip, color filter (CdS film), APD photo sensor; moreover, the related characteristics analysis on Si substrate will be performed in order to build the key technologies related to the low temperature growth of micro biological and medical photo sensor device.

In addition, since the fluorescence signal need to be measured in sub-project 4 is very weak, the photo current generated by photo detector diode is going to be very weak too, how to overcome the effect of noise on the signal and how to detector the change due to such weak signal are all the challenges in this project. In order to reduce the effect of noise on real signal and to reduce the system circuit volume, this project is going to complete simultaneously the design and development of the integrated circuit for a high sensitivity photo detection system single chip in order to process the signal sensed by the photo sensor device.There are three main parts in this integrated circuit（1）Low noise amplifier(LNA) (2)High resolution analog digital converter(ADC)（3）Digital control and display circuit(Controller). The whole structure of the single chip is as shown in figure 4.

The LNA and ADC circuit parts used in the single chip are analog circuits, the controller part is digital circuit, therefore, the development of the single chip integrated circuit is actually a circuit of mixed mode signal and highly challenging. This project is going to complete LNA, 16~18 bit ADC and part of the circuit design of Controller, among them, the LNA and ADC will complete its verification through chip preparation, and the Controller part will complete its verification through FPGA, after the completion of verifications, these three parts of circuit will be integrated to form an overall integrated circuit design, finally, its function will be verified through the chip preparation process, this single chip is going to be combined with optical detector part in order to test its sensitivity and its anti-noise capability. In addition, the controller circuit in this project should be finished together with other sub-projects in order to complete the build up of internal information database and the signal differentiation, finally, the result will be displayed on a LCD screen in order to provide information

regarding to whether the aquaculture original illness-causing bacteria is existing or not or information such as its quantity of existence.

6.

Professor Chung-Cheng Chang, the leader of the overall project as well as sub-project 3, has his expertise include optoelectronic, integrated circuit processes, nano and micro Electro-Mechanical-Systems, sensor device system and under water technologies, etc.; his research direction include the preparation and application of nano and micro integrated sensors. His main study focused on MEMS, optoelectronic and IC technology integration for the preparation of integrated sensor device. He has more than 20 years of experiences in the research of optoelectronic devices since his entrance into this university in 1982. He started with the preparation of integrated sensor device by using MEMS, optoelectronic and IC technologies in accordance with the university's development goal, he has accumulated more than 15 years of experiences in these fields and completed the preparation and testing of integrated devices such as: Si pressure sensor, infrared array sensor, ultrasonic sensor and short wavelength optical sensor, etc., these devices are applied in the temperature and infrared sensing, blood pressure, heart beat, short wavelength optoelectronic sensing, ultrasonic distance measurement, ultrasonic distance imaging, etc., his expertise make him so qualified to integrate the research of Lab-on-a-chip.

He is the electrical engineering department chief in 1992-1995 and has the experience of leading an integration type project, they are as in the followings(a) Technical development and preparation of smart underwater mechanical arm system –overall project；sub-project 1: Distance measurement and technical development of smart underwater mechanical arm system, (b)Technical development of the application in the underwater operation of the remote control underwater mechanical arm system, (1)-overall project；sub-project 1: Technical development of the distance measurement technology applied in the underwater operation of the remote control underwater mechanical arm system, (c) Development of underwater sensor device and system and its application in underwater operation-overall project (1/3)；sub-project 1: the development of underwater piezoelectric ultrasonic array system and underwater sensor device electronic support system, etc.

He has experience helping a seminar sponsored by The Electronics Devices and Materials Association held in this university in 2003, he was the agenda chairman. He is also the vice group leader and group leader of the North MEMs research center. He was also the sub-item chairman of the 2004 International Symposium on Underwater Technology; UT'04. In addition, I have spent lots of efforts on helping and promoting the modification of nano and micro technology courses of this university and the planning of regional nano human resource training center sponsored by the Ministry of Education, I am very confident on project integration and coordination.

This research group has started the development of Lab-on-a-chip platform technology co-lead by me and professor Po-Tai Chen and professor Sheng-Yu Chao, currently, we have initial result on microchannel system and fluorescence detection system, in accordance with the build up of Lab-on-a-chip platform and the development of portable Lab-on-a-chip detection system for aquaculture original illness-causing bacteria detection, we need to invite more professionals such as: professor Chao-Te Chen in the Aquaculjture Department, professor Chih-Wei Wu in the Mechanical Engineering

Department and professor Wan-Jung Liu and Chih-Chieh Ko in the Electrical Engineering Department. The specimen preparation and process flow planning by professor Chao-Te Chen has now come to an initial completion；the microchannel system preparation by professor Po-Tai Chen, Sheng-Yu Chao, and Chih-Wei Wu has produced initial microchannel specimen; professor Chih-Chieh Ho is in charge of the preparation of optical sensor, currently he has initial result on Si-Ge optoelectronic sensor；professor Wan-Jung Liu is in charge of the data processing and the preparation of LCD monitor IC, he has now some initial design results；I am in charge of integrating microchannel system and fluorescence detection system for Hybridization and optoelectronic detection；currently, initial build up of fluorescence detection system has been completed, real specimen measurement has been completed in accordance with professor Chao-Te Chen, the Lab-on-a-chip platform can be completed after integrating the assembled sensor and microchannel system.Since the development of Lab-on-a-chip detection technology needs professional with chemistry as well as software background；we therefore invite Dr.Chih-Yen Cheng in Academia Sinica and professor Jung-Hua Wang in the Electrical Engineering Department to join our research group. Currently, the expertise of all the group members can cover all the detailed parts of this project, we thus has completed a basic job assignment architecture, part of the integration work is on the way, the research and development result can be extensive if this integration project can be executed.

7.

Many professors in the College of Life and Resource Sciences have focused on the study of aquaculture species and the results is so fruitful, we have very complete related research equipments; for instance, the leader of sub-project 1 of this project, professor Chao-Te Chen is from Aquaculture Department, his expertise is in molecular biology technology, he is very experienced in the bacteria detection research field. The whole aquaculture original illness-causing bacteria detection process flows can be completed in his lab, therefore, there is no problem in the design of the whole LOC chip detection flows. In other sub-projects, the LOC microchannel system and fluorescence detection system can be prepared according to these process flows in order to perform the rapid detection of original illness-causing bacteria. In the LOC expertise aspect, we have resources from Electrical Engineering Department, System Engineering and Naval Architecture Department and Institute of Material Engineering from the College of Engineering, we have Mechanical Engineering Department and Marine Engineering Department from College of Maritime Science, as well as Institute of Optoelectronic Sciences from the College of Science. In the recent years, the university has promoted nano and micro Electro-Mechanical-Systems technology, the nano and micro technology course has been set up, it attracts the attentions from many students; meanwhile, the nano and micro Electro-Mechanical-Systems lab has been built up.Since the university provides so strong support to the research in the nano and micro technology field, it also encourages the professors in the related fields to apply for nano and micro biotechnology projects and gives promise to support corresponding project fund. Besides, this university has very good experiences and resources in the professor manpower and lab equipments in the field of the preparation of microchannel system and fluorescence detection system. Accompanied with the research results in the aquaculture species, we are standing at the advantages of

plentiful research resources. In the equipment aspect, we have complete resource for biological preparation; in the device preparation aspect, for large equipments, we can borrow them from Nano-Electro-Mechanical-Systems Research Center in National Taiwan University（hereafter will be abbreviated as Research Center, it emerges from former North Micro-Electro-Mechanical-Systems Research Center, National Science Council）, Opto-Electronics and Systems Laboratories in Industrial Technology Research Institute and NDL. For general equipments, they are available in the nano and micro Electro-Mechanical-Systems lab and the labs in the related departments in this university, therefore, the purpose of full use of the equipments in this university and the regional center equipments in the Research Center is then achieved, it also promotes the exchange and interaction in terms of domestic nano and micro technology. Nano and micro Electro-Mechanical-Systems manufacturing technology is the key technology expected to be developed for the aquaculture original illness-causing bacteria detection chip system in this project, in addition to the use of the nano and micro Electro-Mechanical-Systems labs which are built ready by this university, the biochip research group in National Taiwan Ocean University has very close relationship to the Research Center. The chief leader of this project, professor Chung-Cheng Chang, is now the academic and technological integration group leader, he has frequent involvement with the affairs in the Research Center, the co-leader of the sub-project, assistant professor Chih-Wei Wu has been in the post doctoral position in the Research Center for four years, he is in charge of some key jobs such as: the operational management, research and development, cooperation between academy and industry, educational training, safety and environmental protection in the Research Center, he spent great efforts on the developing and planning of the Research Center, his support is so helpful to the smooth execution of this project. Besides, the Soft Computing Lab. provide great support on the software; the preparation and test of microchannel and hybridization chamber is supported by Dr. Chih-Yen Cheng in Academia Sinica.

8.

1. Completely develop portable prototype lab chip for the detection of aquaculture original illness-causing bacteria.
2. Develop highly precise and sensitive biochip prototype in order to detect the original illness-causing bacteria in the aquaculture pond such as:*A. hydrophila* and *E. tarda*.
3. Develop unique probe detection chip with advanced multiple microchannels in order to detect simultaneously the commonly seen and known original illness-causing bacteria in the aquaculture pond to meet the actual application requirements for the aquaculture pond owners.
4. Build micro filtering system for replaceable specimens and install automatic sampling mechanism to achieve the automation of analysis and detection.
5. Develop micro fluid system devices such as micro pumps, micro valve, micro mixer and micro channels.
6. Complete the operation flows for the release of RNA(ribonucleic acid)from the dissolved cell in the microchannel system of the lab chip.
7. Complete the operation flows for the purification of RNA(ribonucleic acid)from the

chromatography column in the microchannel system of the lab chip.

8. Complete the experiments performed in the centimeter scale flow channels, meanwhile, use the mass point image speed measurement method to measure the mixing and enhancement effect of the bubble breakage flow field on the low Reynold Number flow field.
9. Generate and break the bubble in the microchannel and use the micro mass point image speed measurement to measure the mixing, flushing and separating effect under microscope, the result is going to be used as a reference for the future improvement on the preparation of microchannels.
10. Build the manufacturing processes of micro array tin film for hybridization and the processes of embedding them in the microchannel hybridization and detection chamber.
11. Build fluorescence detection model for the specimens used in this project and complete the integration of fluorescence detection system so that the specimen after hybridization can be fluorescence-detected using fluorescence detection system in the hybridization and detection chamber.
12. Complete the quantization standards for the fluorescence detection signals.
13. Complete the integration of micro flow system, fluorescence detection and the display system, build a complete aquaculture original illness-causing bacteria detection lab on a chip(LOC)system.
14. **Complete** the study of **Poly-$Si_{1-x}Ge_x$** of different compositions based on MILC technology on characteristics such as growth temperatures, horizontal crystallization rate, crystallization particle size and resistance curve(I-V curve)；meanwhile, Au is designed to be used as a structure for the SiGe/Si epitaxy interface based on MILC.
15. **Complete** the study of **Poly-$Si_{1-x}Ge_x$**/porous Si of different compositions based on MILC technology on characteristics such as growth temperatures, horizontal crystallization rate, crystallization particle size and resistance curve(I-V curve); meanwhile, Au is designed to be used for the SiGe/porous Si epitaxy interface (based on MILC)structure change study, in the mean time, it is applied to the fluorescence detection system.
16. Complete the design and layout of low noise signal amplifier and high resolution analog digital converter and have them produced in the lab. For the digital controller and display circuit, their overall digital circuit design and verification is completed by FPGA, meanwhile, they are integrated with fluorescence detection.
17. Complete the circuit test, verification and circuit correction of LNA and ADC as well as the design and preparation of the whole circuit system, meanwhile, they are integrated with fluorescence detection.
18. Union test and set up of the single chip system circuit and overall biological detection system, in addition, data buildup and analysis are under way to enhance the detection precision.
19. Build the preparation flows for the micro detection system of aquaculture original illness-causing bacteria.
20. Build the operational procedures for the micro detection system of aquaculture original illness-causing bacteria.

9.

The major equipments needed in this project include three parts 1. Equipment for aquaculture biotechnology. 2.Equipment for LOC microchannel system. 3. Equipment for LOC fluorescence

detection system technology, they are described respectively in the followings.

1. The necessary research equipments for aquaculture biotechnology include the cell grinding machine, polymerized enzyme chain reaction equipment, electrophoresis equipment and the fluorescence detection equipment in the labs of Aquaculture Department of our university.

2. The necessary research equipments for LOC microchannel system include lithography machine, evaporation machine, sputtering machine, thermal evaporation machine, LPCVD, PECVD, O_2 Plasma, photo resist spin coater, furnace and ICP, etc. Research center, Precision Instrument Development Center, Semiconductor Center in National Chaiotung University, and the MEMs lab in our university will provide the necessary equipments for the experiment. The photo mask part will be consigned to NDL.
3. The necessary equipments for LOC fluorescence detection system technology include chemical cleaning tank, sputtering machine, VPE epitaxy equipment, oxidation and diffusion equipment, evaporation machine, exposure equipment, resistance analyzer, I-V tester, electrical property measurement equipment; in addition, the laser fluorescence detection system is available in the nano and micro integrated sensor device laboratory. For opto-electronic devices measurement equipments such as: high temperature furnace, C-V tester, thickness measurement equipment, optical mass spectrometer, atomic force microscope, etc., they are available in the Opto-Electronics and Systems Laboratories in Industrial Technology Research Institute and the Institute of Optoelectronic Sciences in our university. Moreover, for ultra low noise signal generator, ultra low noise power supply, high frequency oscilloscope, logic analyzer, etc., they are available in AsicLab in our university. And the photo mask is consigned to NDL.