Thesis for the Degree of Master of Engineering

The design and application of embedded systems in biomedical engineering

By

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Pukyong National University

May 2019

The design and application of embedded systems in biomedical engineering

(의공학에서 임베디드 시스템의 설계 및 적용)

Advisor: Prof. Junghwan Oh

by

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Abstract

Biomedical engineering is an application of engineering methods and design concepts to medicine and biology for health care purposes. In the past, health care purposes mostly depend on biology and chemical methods. But nowadays, with the advance of technology, scientists and engineers seek to close the gap between engineering and medicine. Combine the design, problem-solving skills, and medical biological sciences to develop health care treatment, including diagnosis, monitoring, and therapy. Meanwhile, embedded systems have also proved its important role in a vast of fields: information technology, telecommunication, electronics, … and nowadays is biomedical engineering applications like Scanning Acoustic Microscopy, High Intensity Focused Ultrasound system, ECG, EEG… Most embedded systems have microcontrollers as its core (.i.e. CPUs with integrated memory or peripheral interfaces). So they have a very small size compare to their capability. The develop of embedded system in biomedical engineering will also open a huge opportunity for the advance of public health care, where people can easily possess personal health care system or device as an acceptable price. In the scope of this thesis, the process of design a few systems for biomedical purposes will be described.

Keywords: embedded system, biomedical, mechanical, electrical

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Chapter 1. Introduction

1. Embedded system overview

A combination in which all of its units assemble together following to a set of rules is called a system. It can also be defined as a way of working by doing one or many tasks according to a fixed plan. For example, television is a color displaying system (Figure 1). Its components follow a set of rules to capture the signal from the distributor and display video, sound on the screen. In one system, all of its subcomponents depend on each other. If one of its parts fails, the television may stop working or malfunction.

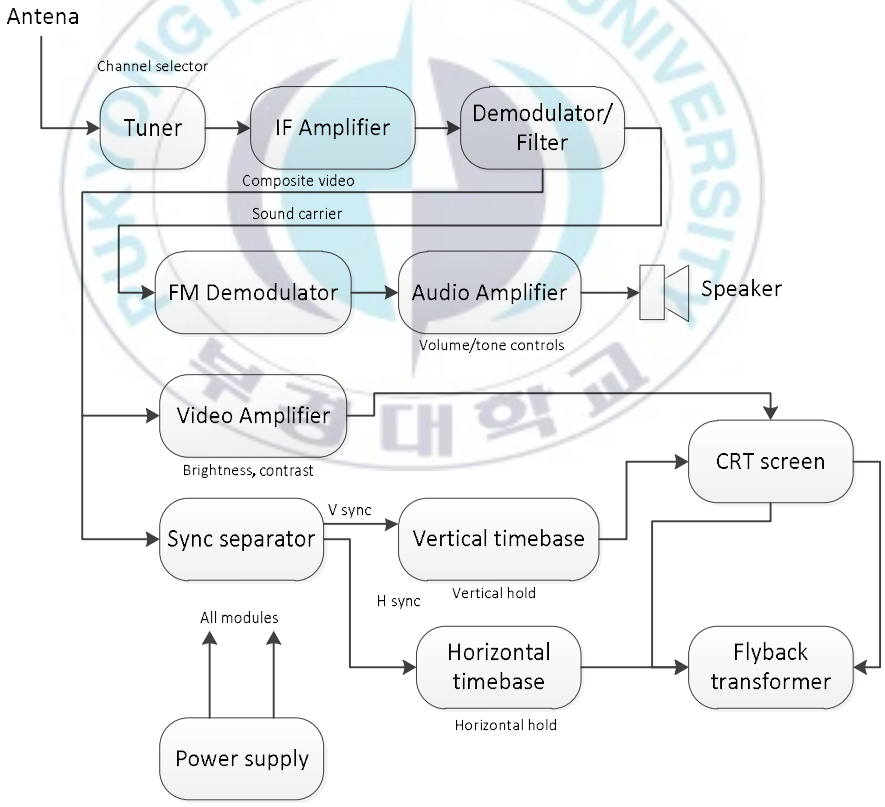


Figure 1: CRT television system block diagram

Embedded system can be thought of as a hardware system having software embedded in it. An embedded system has inherited nature, so it can be an independent sub-system or combined together to form a larger system. For example, a fire alarm system is an embedded system that will sense only smoke and automatically ring the alarm. But it can also combine with a water distributing system to extinguish the fire. Both of this system can work individually, but they can also be combined to work more effectively.

A basic embedded system (Figure 2) based on Microcontroller and must have three components: hardware, application software and Real-Time Operating System (RTOS). RTOS is the way that system works. However, a small scale embedded system does not need to have RTOS. A complete embedded system must have these following characteristics:

- Single-functioned: An embedded system always performs some specific tasks repeatedly. For example, a radio always functions as a radio, not a fan.
- Connected: It must have peripheral communication protocols to connect with input and output devices.
- Tightly constrained: Every computing systems must have constraints on design metrics, but it's even more tightly on an embedded system. Design metrics is a measure of features such as cost, size, power, and performance.
- Memory: It must have memory, known as ROM (Read Only Memory), to store the operating program, or flash memory, to store the data.
- Microprocessor based: It must be microprocessor or microcontroller-based, which control and collect data from input and output devices.
- Reactive and real-time: Many embedded systems must react to the change of environment continuously and compute the response in real time.

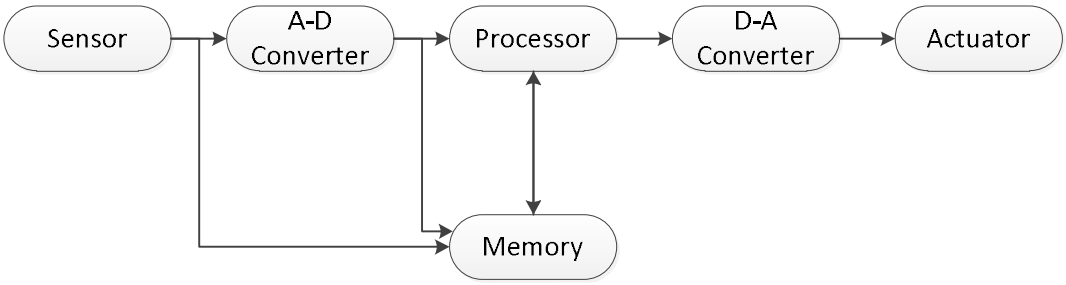


Figure 2: Basic structure of an embedded system

2. Biomedical engineering overview

Biomedical engineering (BME) is the engineering principles that follow the concept of medicine and biology to design applications for healthcare purposes. By combining the design and problem-solving skills of engineering with medical biological, it will create high-value industries such as the development of medical devices and artificial organs as well as fight off the diseases. The growth of BME will definitely provide a high quality of life to human and save millions of lives from diseases such as cancer. Specifically, the field of biotechnology aims to develop various diagnostic and therapeutic devices that can be ready to be used in hospitals. Engineers in this field conduct research on a variety of fields. Concentrations include development of artificial organs such as artificial hearts and ears, processing of vast amounts of patient and gene information, brain engineering, often referred to as the blue chip of the 21st century, where the biomedical and medical device field provide information useful for diagnosis by detecting various bio-signals generated in the human body, and image capturing and processing and analysis methods in the medical field. As such, a biomedical engineering major will directly contribute to the improvement of health, welfare, and quality of life, by cultivating experts that have

both medicine and engineering knowledge. Biomedical engineering is the application of engineering in the fields of biology and health care. Biomedical engineering has developed many technologies that save and enhance life, which are:

- Replacement of body parts like limb or denture.
- Robotics arm and laser surgery devices for surgical.
- Devices that can be implanted inside the body, such as insulin pumps, and artificial organs, heartbeat assist device.
- Imaging methods that can be used to observe complex organ structures such as ultrasound, X-rays, particle beams and magnetic resonance.
- Diagnostics devices, such as ECG, blood chemistry, …
- Exercise equipment and wearable device.

With the advancements of science and technology, biomedical engineering has also evolved over the years. Throughout history, many effective devices have been invented to diagnose, treat diseases, to alleviate, rehabilitate or compensate for disabilities or injuries. Biomedical engineering is all about designing and developing medical systems, equipment, and devices. This field requires a lot of in-depth knowledge about electronic, mechanical, biological, etc. as well as knowledge about the application for which it is to be used. For instance, to design a lab-on-a-chip requires knowledge of electronics, nanotechnology, and biochemistry. A biomedical engineer needed to acquire these critical skills include a well-rounded understanding of several areas of engineering as well as the specific area of application. With the rapid development of technological and potential new applications, continuing education and training are necessary to keep up with the pace.

Chapter 2. Fast Scanning Acoustic Microscopy

1. SAM system overview

Scanning acoustic microscopy (SAM) is a non-invasive technique that can be used by technicians to image the internal features of a specimen. It has been invented in 1974 by R.A. Lemons and C.F. Quate at the Microwave Laboratory of Stanford University. Since then, many improvements have been made to enhance the resolution, accuracy and scanning speed. Overall, SAM is an efficient tool for evaluating sealing quality, printed circuit boards, and applications in biomedical.

SAM method is based on ultrasound. Ultrasound is sound waves that have a frequency higher than the upper limit of human hearing range. Physically, ultrasound has the same physical properties as normal sound, except that humans cannot hear it. Ultrasound devices can be operated with frequencies from 20 kHz up to several GHz. SAM operates using the pulse reflection method. Thus the transducer is the acoustic objective-centerpiece of the microscope. The transducer produces, transmits and receives, then converts short ultrasound pulses to electric signals. In the sample, the sound gets reflected on material interfaces according to the mismatch in acoustic impedance. The transducer receives the sound pulses reflected from the sample and transforms them back into electromagnetic pulses which are recorded as a function of time (A-scans). Analysis of an A-scan result in a pixel with defined gray values. To produce an image, the acoustic objective scans the sample point by point and line by line (Figure 3).

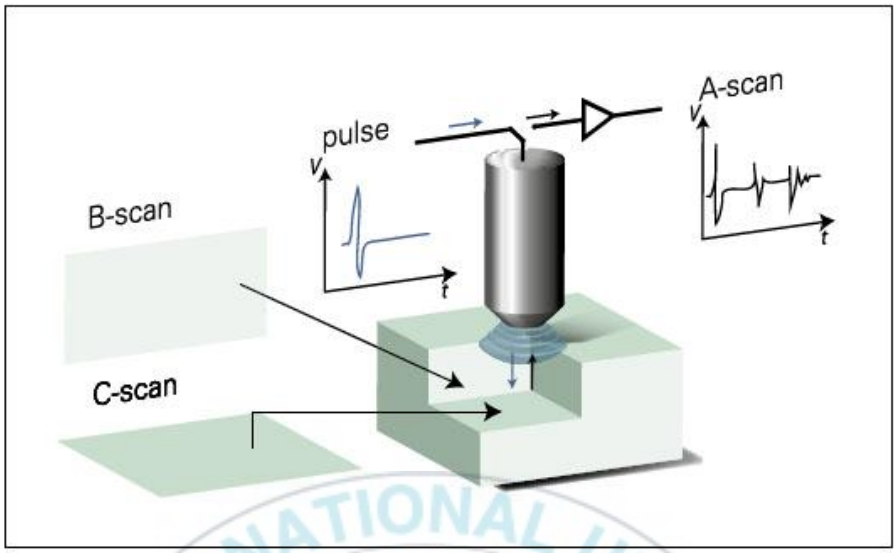


Figure 3: SAM scanning technique

Because SAM systems image the sample by the use of sound energy, samples have to be submerged in a liquid medium to ensure the ultrasound waves propagate to and through the samples. SAM system principal to produce an image is moving an ultrasonic transducer mechanically in a 2D pattern over the test specimens. This scanning technique is usually performed in a liquid tank filled with deionized water or another type of liquid that can couple the ultrasonic energy from the transducer. The transducer emits a short ultrasonic pulse, then receives and converts the echo to electrical signals. If the part has a solid body and free of defects inside, there will be two signals from the near and far surface. If there is an internal problem inside the sample, such as void, the transducer will return additional signal. This electric signal has a relationship directly to the depth of the defect, and the transducer frequency is fixed by the manufacturer. In conclusion, to increase the speed of scanning, the mechanism to move the transducer in 2D planar face need to be fast enough.

2. Mechanical design and data acquisition process

SAM system principal to produce an image is moving an ultrasonic transducer mechanically in a 2D pattern over the test specimens. However, in order to archive higher resolution and greater accuracy, the speed of the scanning is relatively low. To increase the scanning speed, a few methods were made in this study.

Inspired by Ultrasound Bio Microscopy (UBM), which introduced in 1990 Princess Margaret Hospital, Canada. This technique has been applied to handheld devices to scan for a small subject like eye retina. Its principal is a small transducer moving continuously in an arc by oscillating movement, and then the image will be shown on the screen in real time (Figure 4). This oscillating technique could be adapted to SAM to increase the scanning speed.

There are 2 basic types of motion in mechanical motion: rotary and linear motion (Figure 5). However, in the practical world, many complicated movements can be archive by combining these basic motions and mechanisms.

Figure 4: Ultrasound Bio Microscopy technique to scan eye retina

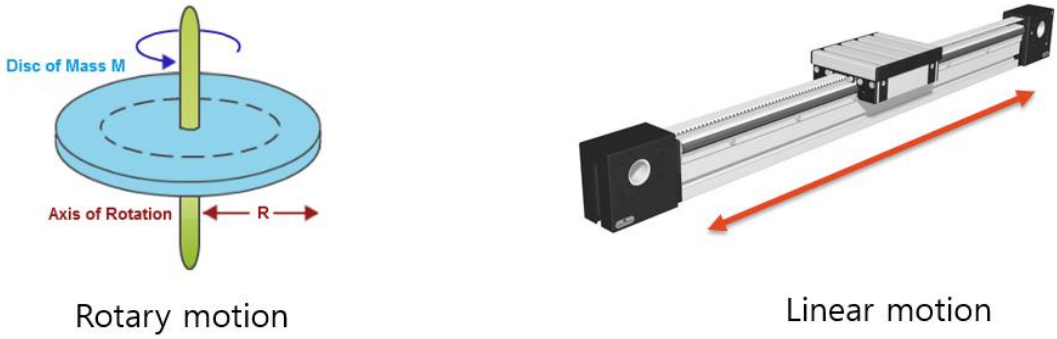


Figure 5: Two basic types of motion

First design is based on peg and slot mechanism, which is shown in figure 6. This mechanism can transform rotate motion from the stepper motor to oscillate movement of the transducer. The cost to fabricate this mechanism is very cheap to implement the scanning acoustic microscopy technique. All parts are made by acrylic material and milled by CNC machine, except the ball bearing part and the stepper motor. In this system, Y and Z axis are controlled by a Gantry robot, while X-axis will be moved continuously by the oscillating mechanism.

Figure 6: Peg and slot mechanism

The sample used for this experiment is 10 Korean won coin (Figure 7). It will be submerged inside the water and scanning by the system in figure 8. To synchronize the movement, the position of the stepper motor shaft need to be known precisely. Therefore, an external encoder is attached behind the stepper motor to monitor the position (figure 9 and figure 10).

Figure 7: 10 won coin sample for scanning, dimension is 22.9mm in diameter

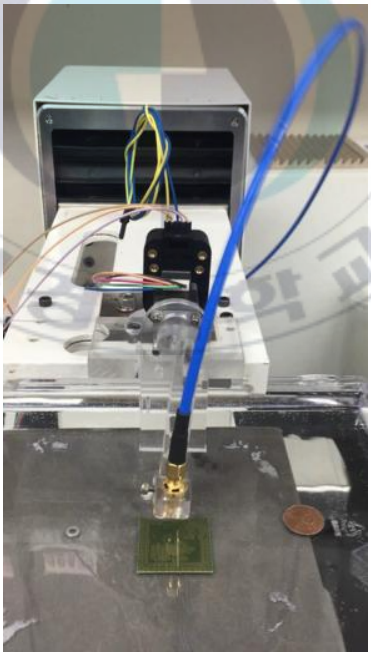


Figure 8: Fast SAM system

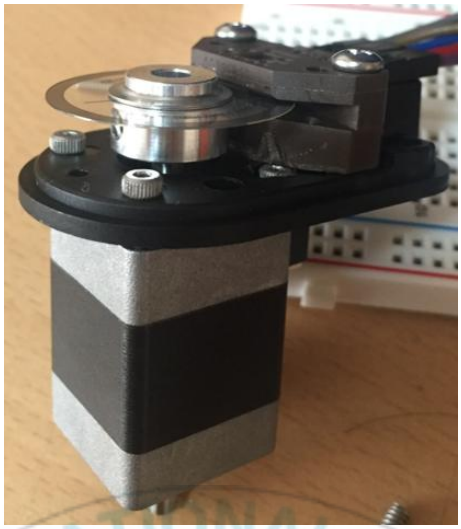


Figure 9: Stepper motor is attached with an encoder

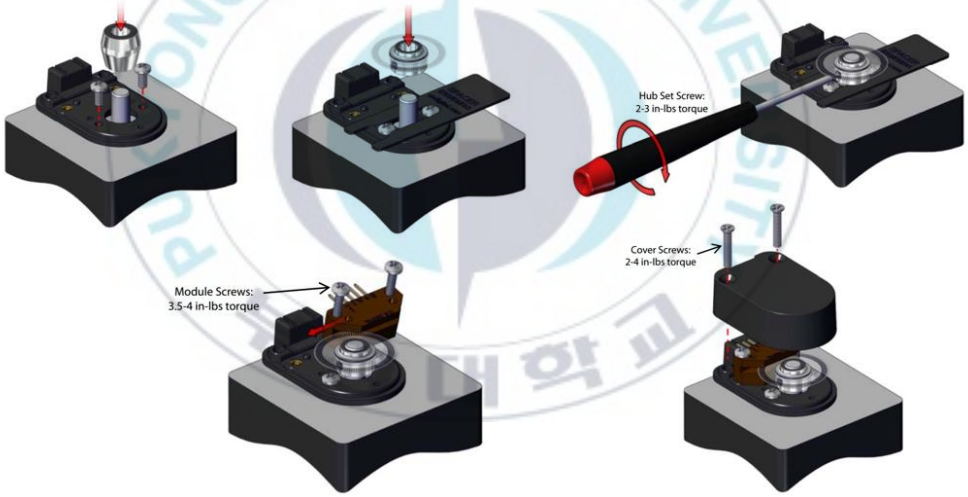


Figure 10: Encoder installation steps

The encoder has 3 channels, A, B and Z (figure 11). Channel A and B are phased shift 90 degrees from each other, used as reference to determine relative position of the shaft. Channel Z (or I) will generate 1 pulse for every revolution used as an absolute position of the shaft. The electric signal from channel A and B will also be used as an external trigger pulse for the transducer. Channel Z is served at a absolute position to define the position of the motor while scanning.

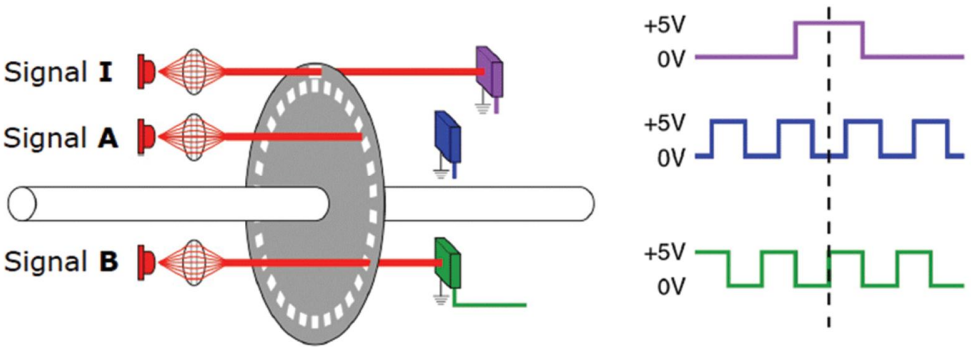


Figure 11: Encoder fundamental

The oscillating motion of the transducer is described in figure 12. In this type of motion, velocity is not always a constant. There are two dwell positions, which has the velocity equal to 0, and the maximum velocity in the middle.

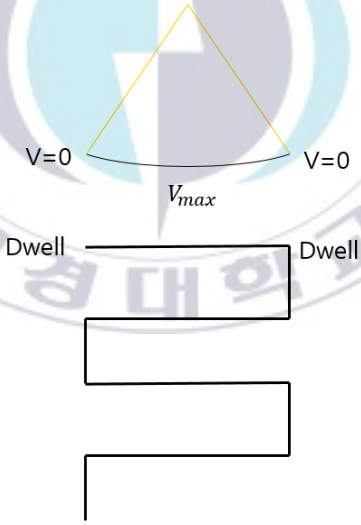


Figure 12: Velocity on oscillate motion along the scanning direction

However, these two different movements need to be synchronized in order to form an image. The method is described in figure 13. X axis is moved continuously by the

oscillating mechanism, while Y-axis is moved by the Gantry robot. And because the velocity of Y-axis is close to 0 near the edge, so a delay of data acquisition is needed

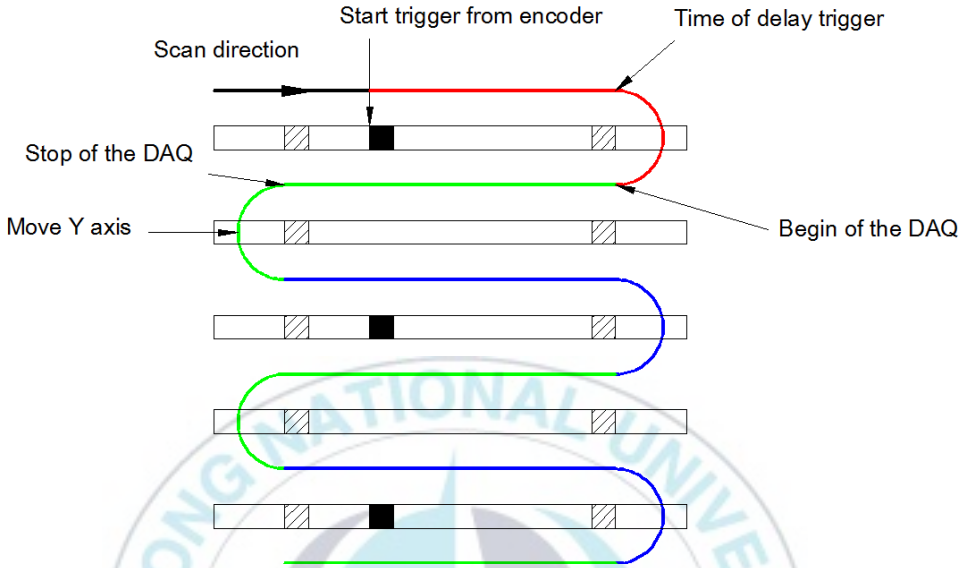


Figure 13: Data acquisition method of the oscillating moving method

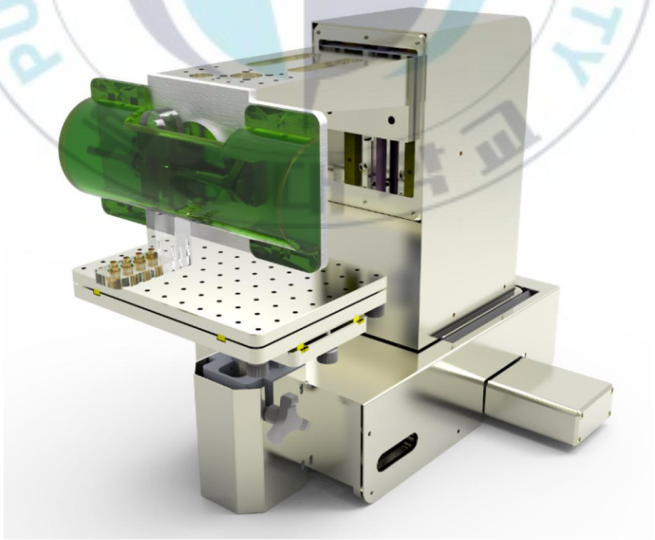
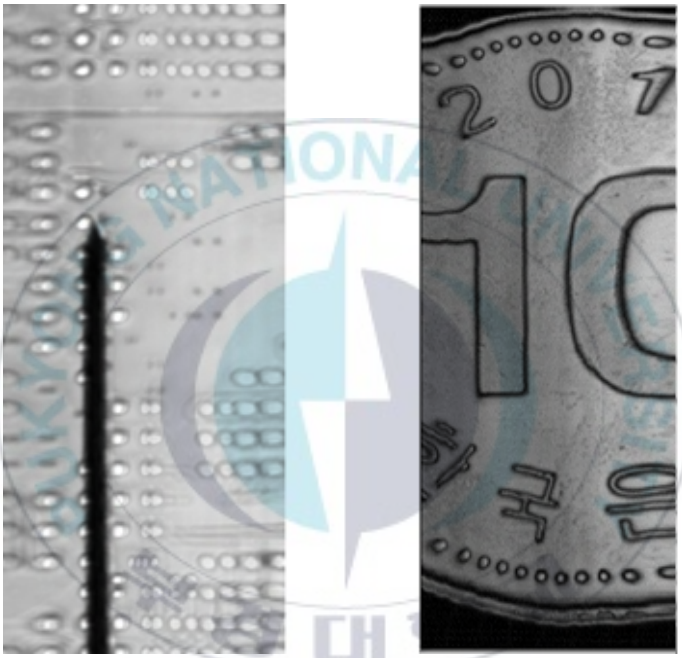


Figure 14: The final structure of this scanning system

3. Result and discussion

In this experiment, a needle and a coin have been used to test the system capability of fast scanning. The scanning area is 16x2.5mm, which is equal to 800 B-Scan (800 lines). The result is showed in figure 15. Compare to the CNC machine, the X-Y axis is driven scanning system, which takes 12 minutes to finish scanning the area, this scanning technique only takes under 1 minute.



a. A needle b. 10 Korean won coin

Figure 15: The result of this mechanism with a few samples.

As the shown result, although the image is clear enough to be recognized the pattern of the coin, or the needle. However, the distribution of pixels on the image is not equally due to the inconstant speed of the mechanism.

The result of this model is still quite modest. However, it is an innovation and base idea for further design that imply fast scanning technique.

Chapter 3: High Intensity Focused Ultrasound system controller

1. HIFU transducer and HIFU system review

Since 1950, high intensity focused ultrasound has rapidly developed for many novel therapeutic applications. The most important application is non-invasive ultrasound surgery, in which a beam of HIFU is focused within the body to raise the temperature of tumor tissues without harming the skin surface. The core of this technique is the HIFU transducer (Figure 16 and 17) powered by an electric system. In this study, 2 MHz commercial HIFU transducer was used, powered by a self-design electric system.

Figure 16: A HIFU transducer

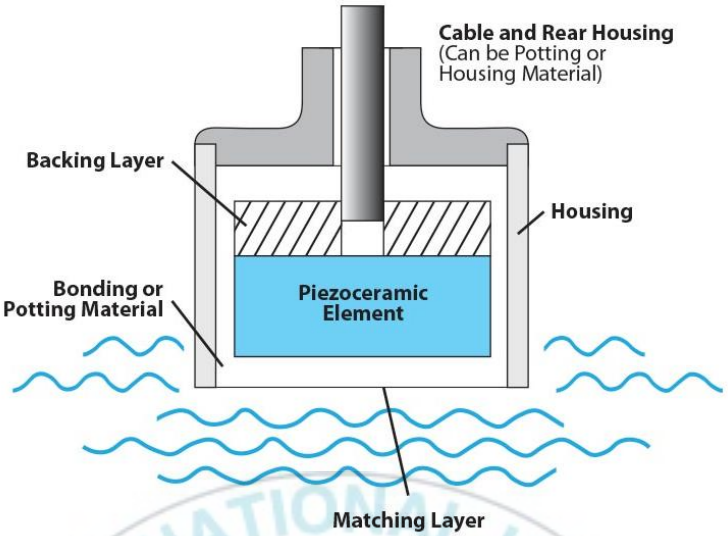


Figure 17: Internal parts of a HIFU transducer

HIFU transducer is based on piezoelectric materials. Piezoelectric materials can change its appearance due to electric current go through it or make electrical signals if its physical appearance is changed. This is also the fundamental of common transducers. If we apply electric pulses to Piezo material, it will vibrate rapidly and create ultrasound. If we increase the amplitude of the pulse, which will also increase the electric power to excite the transducer, Piezo will vibrate with a larger amplitude and has more ultrasonic power. At high ultrasonic vibration, one of its side effect is heating, and HIFU treatment is all about heating. Ultrasound will be focused under the skin of the patient and heat up the unwanted parts, cancer cell for example, to a proper temperature and kill it. While at the skin surface, because the ultrasound is not focused, there will be no heating effect at the surface at all. That is also the reason why HIFU is widely used as a non-invasive surgery method (Figure 18).

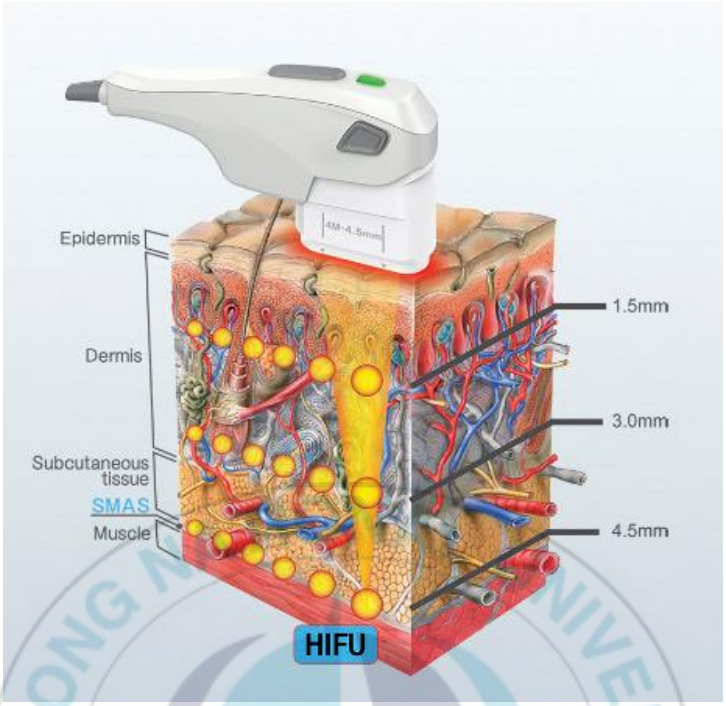


Figure 18: HIFU treatment

2. Electric design

To operate the HIFU transducer, an electric system provides the transducer with high voltage is needed. There are a few parameters that need to be controlled in the HIFU system: Frequency, voltage, current and target temperature.

- Frequency: Every HIFU transducer has a fixed free-oscillation frequency. In the electric system, the frequency of electrical signals needed to be modified to match HIFU free-oscillation frequency. This will create a resonance effect on the HIFU transducer and maximize its output power.

- Voltage and current: These two parameters can be simply implied into one parameter, output power. The higher the output power, the stronger the heating effect of the ultrasound. So for the HIFU system, the output power is very important. There are two different definitions of power, peak power (Figure 19) and average power.

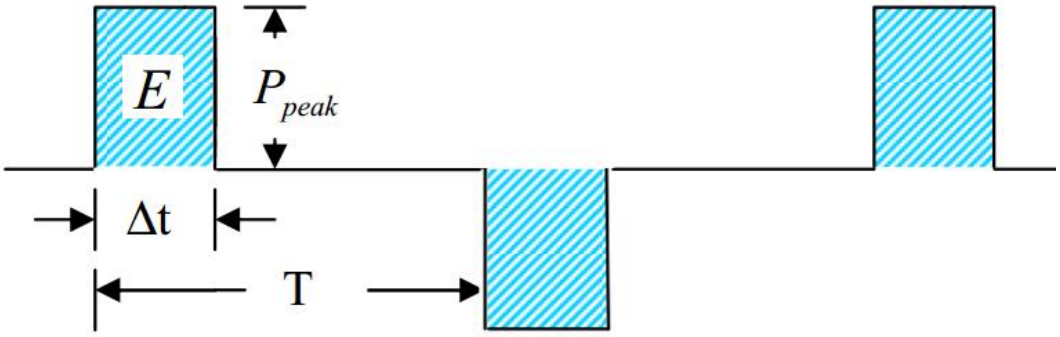


Figure 19: Power distribution in HIFU treatment

Assume the energy contained in every pulse is a constant, E.

Peak power: Rate of energy flow in every pulse

$$P_{PEAK} = \frac{E}{\Delta t}$$

Average power: Rate of energy flow averaged over 1 full period

$$P_{AVG} = \frac{E}{T}$$

We also have:

$$Duty\ cycle = \frac{\Delta t}{T} = \frac{P_{AVG}}{P_{PEAK}} = 50\% \Rightarrow \quad P_{PEAK} = 2P_{AVG}$$

Power of ultrasound is defined as the rate of energy transfer and is measured in Watts. But the key variable in the ultrasound beam is intensity (W/cm^2). The higher the intensity, the faster the temperature will rise. Therefore, peak power is more important than average power.

- Target temperature: HIFU system can kill cancer cell below the skin surface with proper treatment time and temperature. However, if the treatment temperature exceeds the safe thresh hold, normal tissues will likely get destroyed as well. Thus the temperature needs to be monitors in real time for the best result.

In this system, the centerpiece is the Micro Controller Unit (MCU, Figure 20).

Figure 20: Microchip microcontroller

It takes the role as the brain of the whole embedded system, which is controlling sub-system, converting temperature from the thermal couple and peripheral communicate with the Human Machine Interface touchscreen (HMI). The block diagram of the system is shown in figure 21, powered from a power supply adapter 24VDC/8A. After going through the power boost up module, the voltage will be boosted to a higher voltage in order to increase the output power. In the power amplifier module, this high voltage will be paired with pulse signals from signal generator module to form high voltage, bipolar pulses. These pulses can excite the HIFU transducer to make the ultrasound and heat up the target. A thermocouple is used to read the temperature at the target in real time. All of this information will be shown on a touchscreen. In the scope of this study, the controller part will be focused.

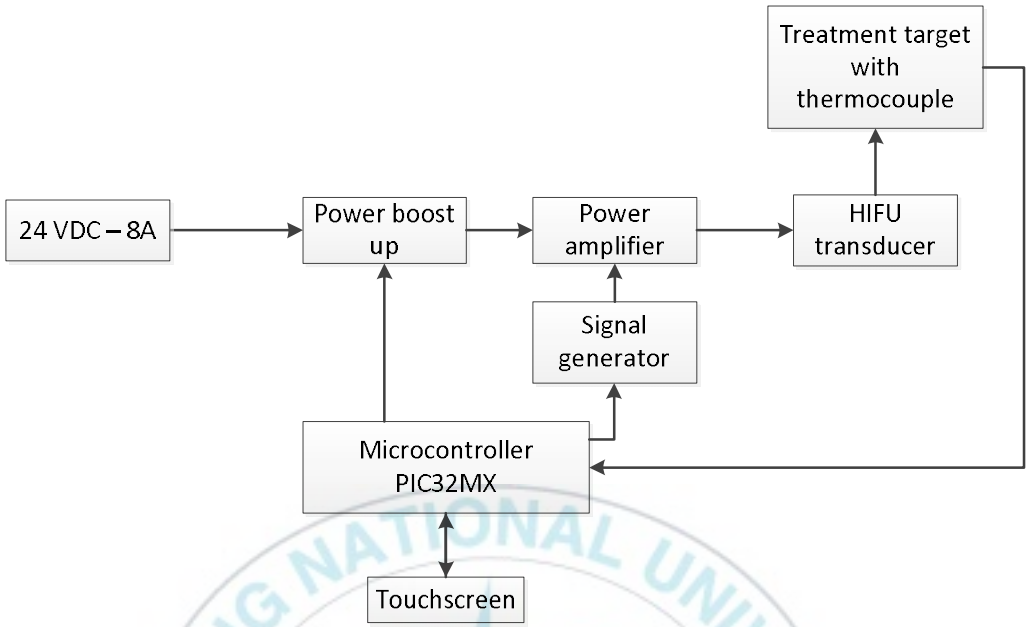


Figure 21: Block diagram of the HIFU controller system

a. Signal generator module

Signal generator module has the role of creating electric pulses with a frequency that match HIFU transducer free-oscillation frequency. And because there are so many types of HIFU transducer with different operating frequency, the requirement for this module is that the frequency of pulses can be changed. For this purpose, a pulser IC has been used (Figure 22). In this LTC6908 IC, there are two channels for output pulses. The frequency of these pulses can be modified by changing the voltage supply to pin R_{SET}.

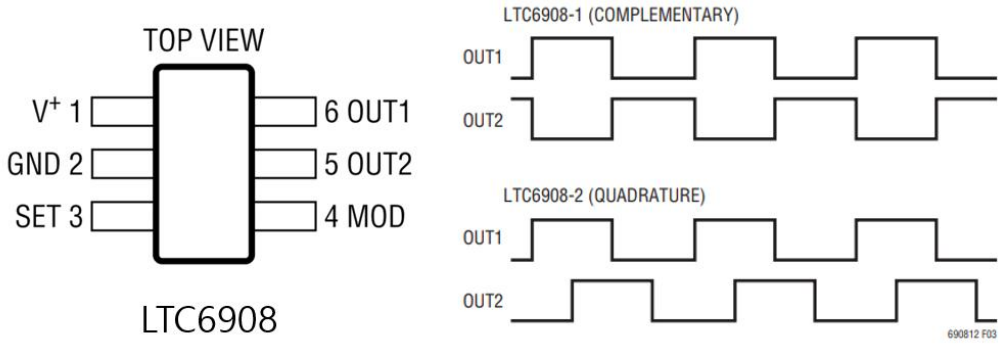


Figure 22: Pulser IC to create high-frequency pulse signals (1 kHz – 10 Mhz)

There are many modes to operate this IC, but in the controllable mode, the output frequency can be changed by changing the current flow into pin SET, this current is changed by changing the resistance of R_{SET} (Figure 23). The equation to calculate the output frequency is:

$$f_{out} = \frac{10k \times 10MHz}{R_{SET}\left(1 - \frac{V_{CONTROL}}{1.13V}\right)}$$

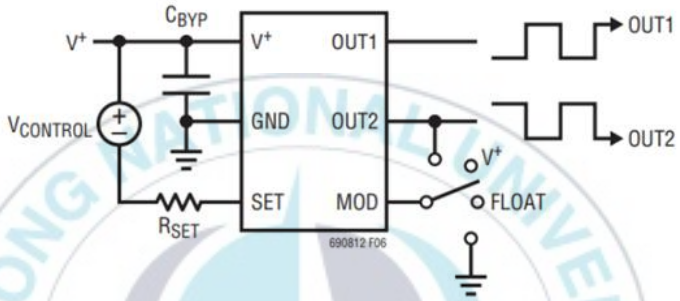


Figure 23: LTC6908 frequency control mode

This resistor is replaced by a digital potentiometer, which can change its resistance by the coding method. This digital potentiometer communicates with MCU via SPI (Serial Peripheral Interface) protocol. SPI was developed by Motorola in the mid-1980. This is a synchronous serial communication that works as a Master-Slave structure (Figure 24). In this structure, MCU is the master, sending commands to the slave, digital potentiometer. They are connected together via 4 wires:

- SCLK: Serial clock (Output from master)
- MOSI: Master Out Slave In (Data output from master)
- MISO: Master In Slave Out (Data output from slave)
- SS: Slave Select

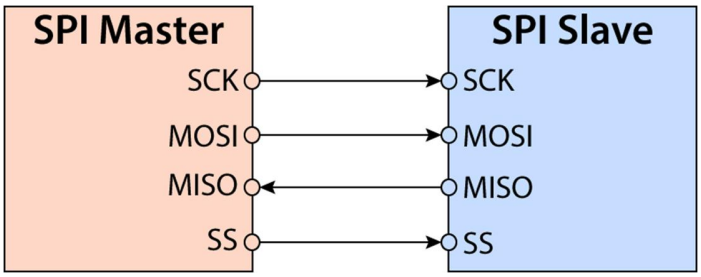


Figure 24: SPI slave-master structure

The connection between MCU and digital POT is shown in figure 25. CS, SCK and SI pins are for peripheral communicate. PA0, PB0 and PW0 pins are 3 pins of the potentiometer.

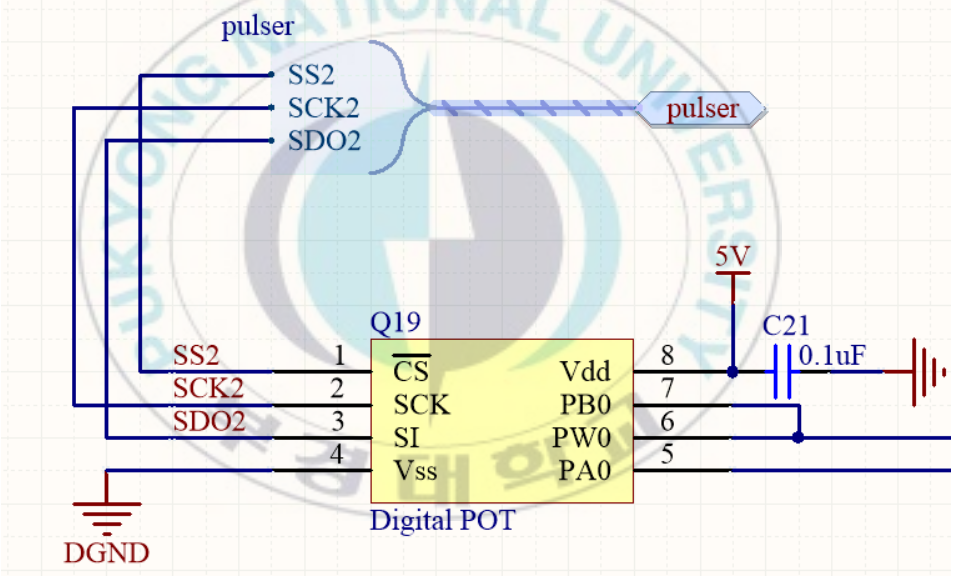


Figure 25: Digital POT connection to MCU

This digital potentiometer resolution is 256 with the maximum resistance of $100k\Omega$. To change the resistance of digital POT, the resolution need to be sent from the master device via SPI. The equation to calculate resolution from the desired resistance is:

$$N = 256 - \frac{256(R - 125)}{100k}$$

b. Thermocouple converter module

To monitor the temperature at the treatment target, a thermocouple has been used. A thermocouple is the junction of two dissimilar metals (Figure 26). When it heated, the heat energy forces the free electrons of one metal to another, thus forming a voltage between them.

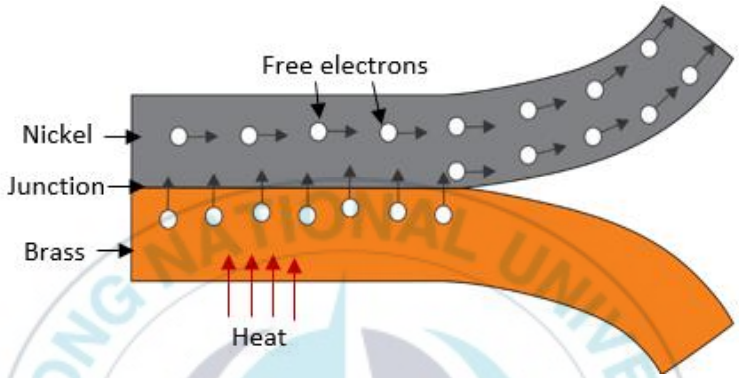


Figure 26: Nickel-Brass thermocouple

Advantage	Disadvantage
Wide range of temperature	Complex signal conditioning
Immune to shock and vibration	Corrosion without protective cover because thermocouple consists of two different types of metal
Rapid response within a few hundred millisecond	Low accuracy compare to another type of temperature sensor
None self-heating because no excitation power is needed	Electrical noise

To measure the temperature, there are two junctions, one junction is set at reference and the other junction can measure temperature based on that reference (Figure 27).

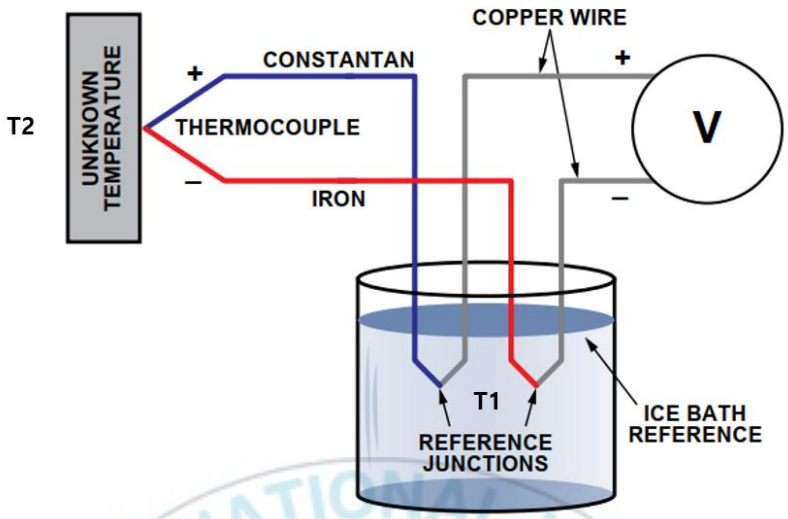


Figure 27: Two junction in thermocouple measurement

Temperature and voltage output is described in this equation:

$$E_{mf} = \int_{T_1}^{T_2} S_{12} \times dT$$

With:

E_{mf}: Electro Motive Force produced by the thermocouple

S_{12}: Thermocouple Seebeck coefficient of two metals

T1 and T2: Temperature at measure point and reference point

From this equation, the voltage is zero when:

- Two thermo-elements make of the same material, so $S_{12}=0$
- T1=T2, which means two junctions are kept at the same temperature

There are several types of thermocouples, which is classified by a couple of metal, temperature range, durability, vibration, accuracy,… In HIFU applications, type K

(Nickel-Chromium or Nickel-Alumel) thermocouple is commonly used because of its inexpensive, accurate, reliable and wide temperature range (Figure 28).

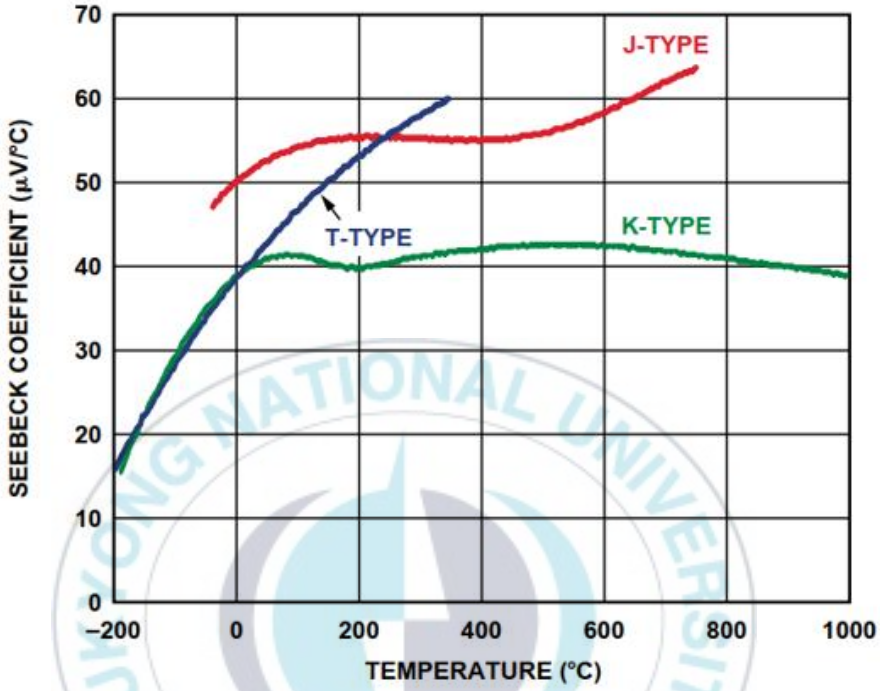


Figure 28: Seebeck coefficient for different kinds of thermocouple

However, the voltage signal produces from the thermocouple is very small. For type K thermocouple, the resolution is $40\mu V/^oC$. This small signal need to be amplified. Thus a high resolution ADC chip integrated with high amplifier has been used (Figure 29)

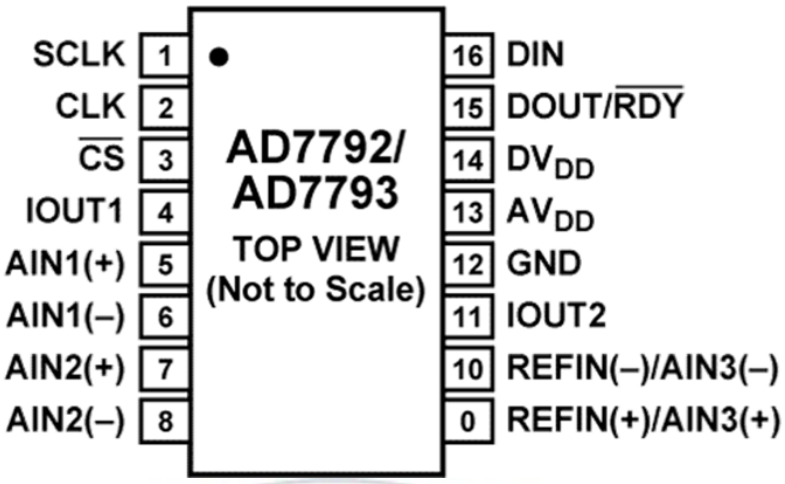


Figure 29: ADC chip AD7793

The design for this IC is shown in figure 30. In this design, the IC is also driven by SPI protocol from the MCU. This IC has 3 channels, data from each channel are 24 bits resolution, which means the maximum data value is 2^{24}. From the datasheet of the IC, we have this equation:

$$Data = 2^{N-1} \times \left(\frac{AIN \times GAIN}{V_{ref}} + 1 \right)$$

With:

Data: 24 bits of data read from ADC IC

N=24: ADC resolution

AIN: Analog input voltage, the voltage generated from the thermocouple (Channel 1) or thermistor (Channel 2)

GAIN: The multiplier of the amplifier, can be set from 1 to 64

V_{ref}: Reference voltage, from this design $V_{ref} = 2V$

$$=> AIN = \left(\frac{Data}{2^{N-1}} - 1 \right) \times \frac{V_{ref}}{GAIN}$$

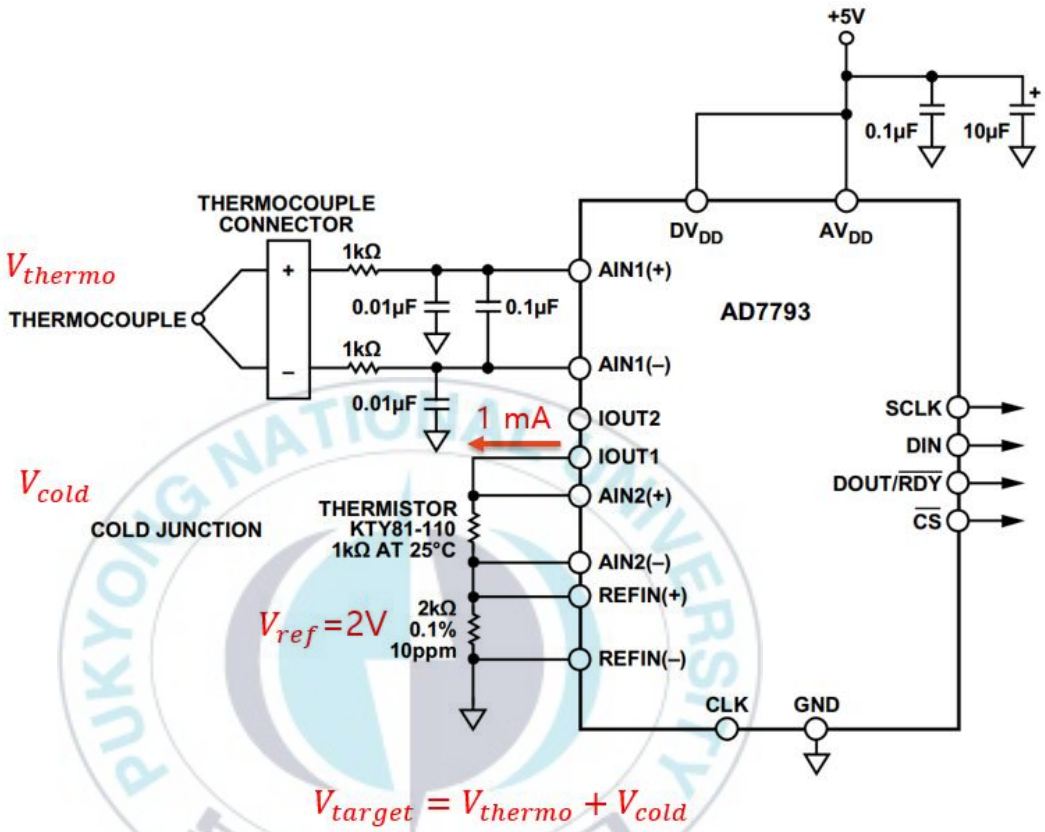


Figure 30: ADC chip schematic design

Channel 1 is connected to the thermocouple connector. For type K thermocouple, the relation between temperature and voltage is close to linear with the ratio of $40\mu V/^oC$. So the temperature read from channel 1:

$$T_{Thermo} = AIN1 \times 40\mu V/^oC$$

Channel 2 is connected to a thermistor. A thermistor is a type of resistor which resistance depends on temperature. From 1 mA excitation current, assuming a linear transfer function, thermistor value varies from $0^oC(815\Omega)$ to $30^oC(1040\Omega)$. This thermistor acts as a reference junction.

$$R = \frac{AIN2}{I_{ex}}$$

$$T_{cold} = 30 \times \frac{R - 815}{1040 - 815}$$

With:

T_{cold}: Cold junction temperature

R: Thermistor resistance

AIN2: Analog input voltage of channel 2, with the gain of 1

I_{ex}: Excitation current, 1mA

And finally, the temperature at measure point will be

$$T_{target} = T_{thermo} + T_{cold}$$

c. Touchscreen module

In the touchscreen module, a Nextion touchscreen (Figure 31) has been used as a communicator between user and MCU.

Figure 31: Nextion touchscreen

This touchscreen uses UART (Universal Asynchronous receiver – transmitter) protocol to communicate with MCU. UART need two pins from each device, Tx (Transmit) and Rx (Receive) connected together to perform the communication process (Figure 32).

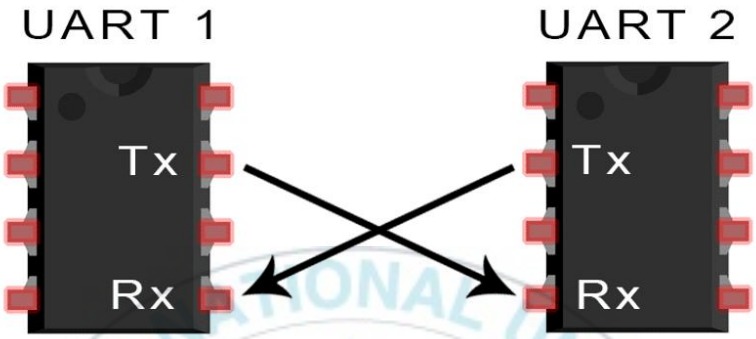


Figure 32: Example of UART connection

In the custom design of this touchscreen, there are 3 pages. In these pages, buttons and indicators were made to interact with the user.

Figure 33: Touchscreen front page

The front page is introducing the application of this embedded system and a button to switch to the second page.

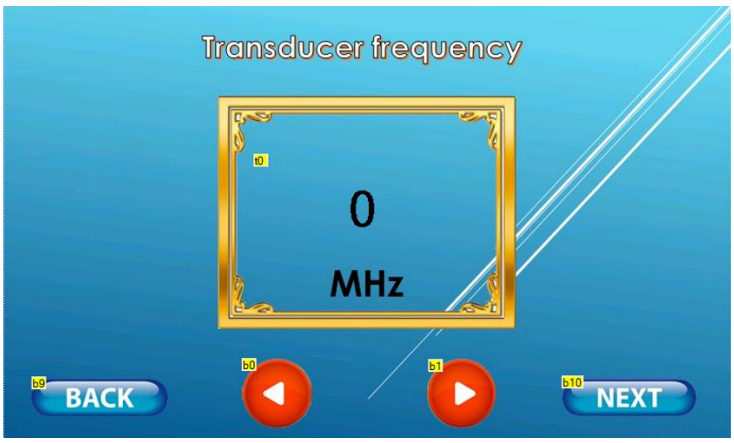


Figure 34: The second page, set the output frequency

As mentioned before, the output excitation pulses from the circuit must be the same as transducer free frequency for resonance effect. In the second page, users can use two buttons to modify the output frequency and observe it in real time.

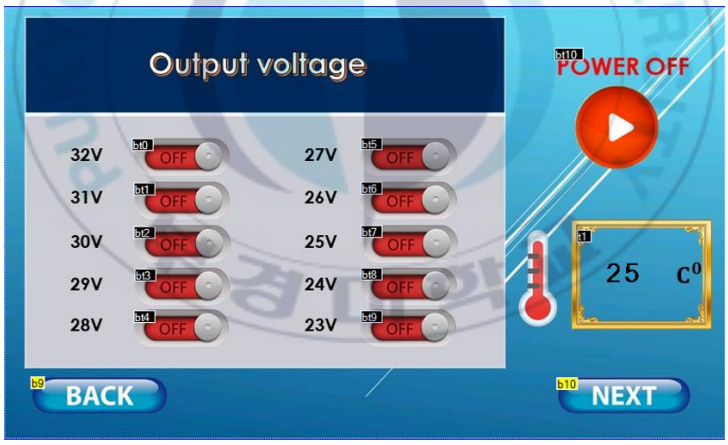


Figure 35: Final page, choose output voltage

In the last page, users can choose the output voltage by flipping the switch, and then press the power button to start exciting the HIFU transducer. Meanwhile, the temperature read from thermocouple will be fed back and indicated directly on the touchscreen.

3. Result and discussion

The PCB (Printed circuit board) for this controller has been designed by Altium. It is a program specifically for designing PCB. Figure 36 is the 3D design of the PCB.

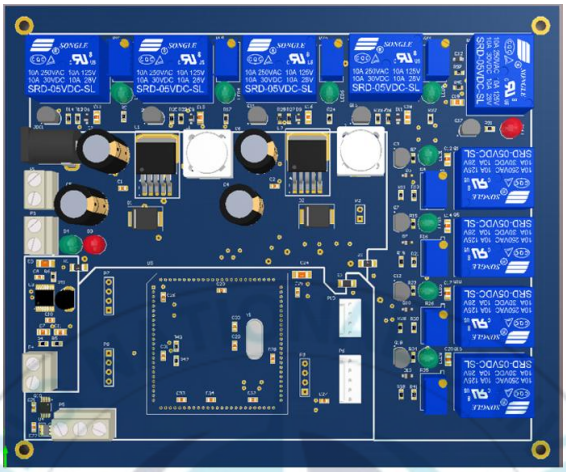


Figure 36: 3D design of the HIFU controller PCB

Figure 37: Controller PCB after soldering all components

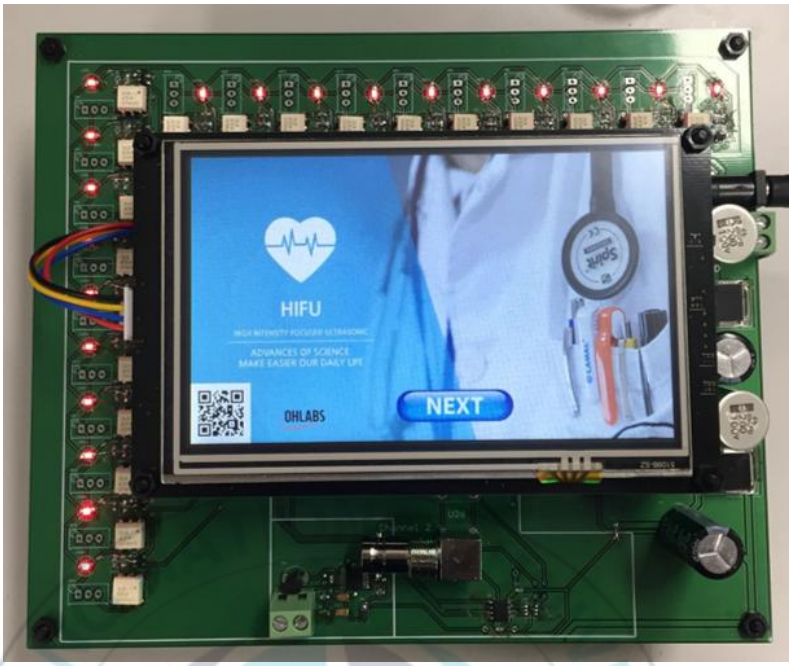


Figure 38: HIFU controller front page

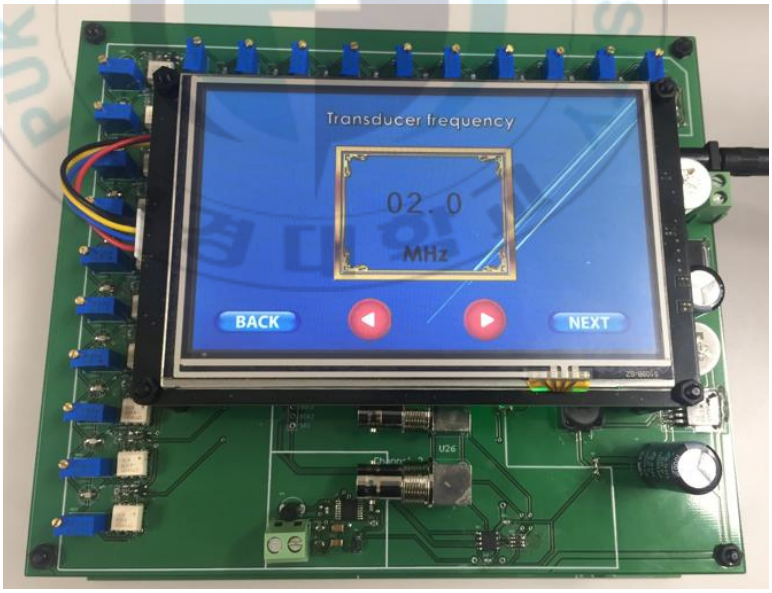


Figure 39: HIFU controller second page with output frequency at 2 MHz

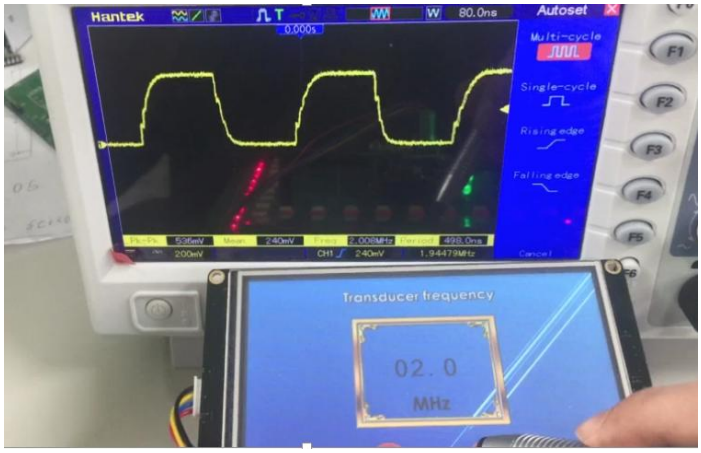


Figure 40: Output frequency measure by oscilloscope

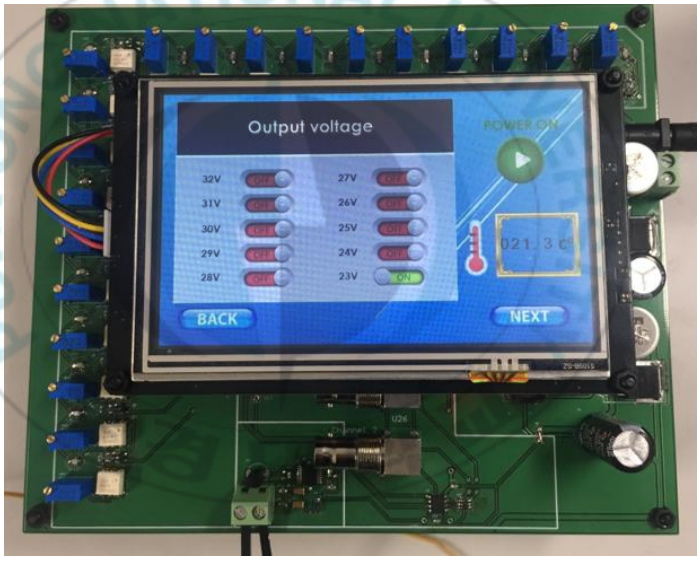


Figure 41: Output voltage set at 23V. Temperature feedback indicate directly on the touchscreen

Chapter 4: Gold-nanoparticle selective detection of breast cancer cell by using Rayleigh Resonant Scattering (RRS)

1. Rayleigh Resonant Scattering and gold-nanoparticles synthesis overview

In the past few decades, metallic nanoparticles have received much attention due to their optical, electrochemical and catalytic properties, which offers enormous opportunities for applications in various scientific and technical fields. In particular, silver (AgNPs) and gold nanoparticles (AuNPs) have been widely employed in the design of chemical sensors for numerous relevant species in the medical, forensic, food safety and environmental fields, including proteins, DNA, toxins, and metallic ions. Special attention has been paid to colorimetric chemical sensors based on AgNPs and AuNPs because of their high extinction (absorption plus scattering) cross-sections within their localized surface plasmon resonance (LSPR) band. In biomedical, one of AuNPs application is that it can be used as nanobullets to kill cancer cell from the inside by using a short laser pulse to trigger Coulomb explosion (Figure 42).

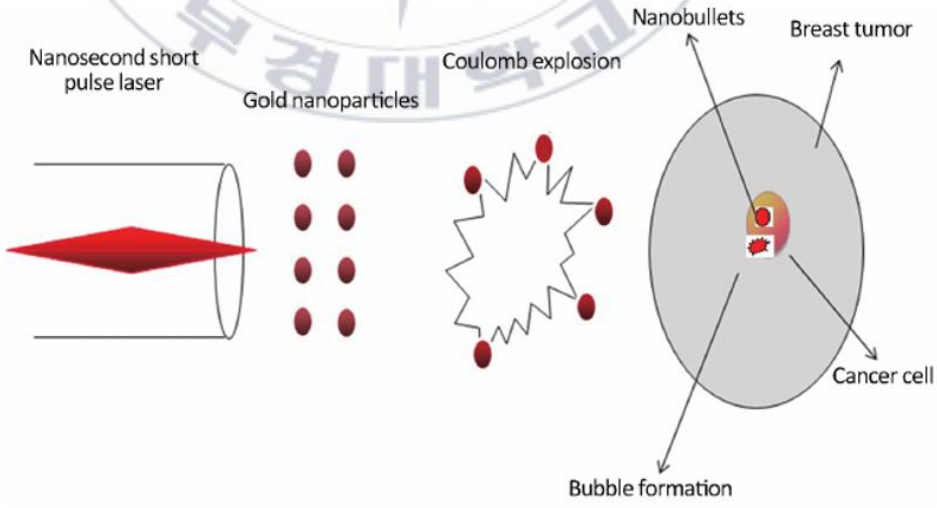


Figure 42: Coulomb explosion kill breast cancer cell from the inside

This method is called targeted therapy. However, in cancer cell treatment, all the dangerous cells must be killed at once or they will grow again. Therefore cancer cells are needed to uptake AuNPs before treatment. A few methods have been introduced to measure this process, one of them is Rayleigh Resonance Scattering (RSS). Rayleigh scattering refers to the scattering of electromagnetic radiation (including light) in a volume of variant refractive indexes. This scattering mechanism is the primary cause that gives us the blue sky (Figure 43). Because blue light with shorter wavelength ($\lambda \sim 500nm$) is strongly scatter than the longer red light wavelength.

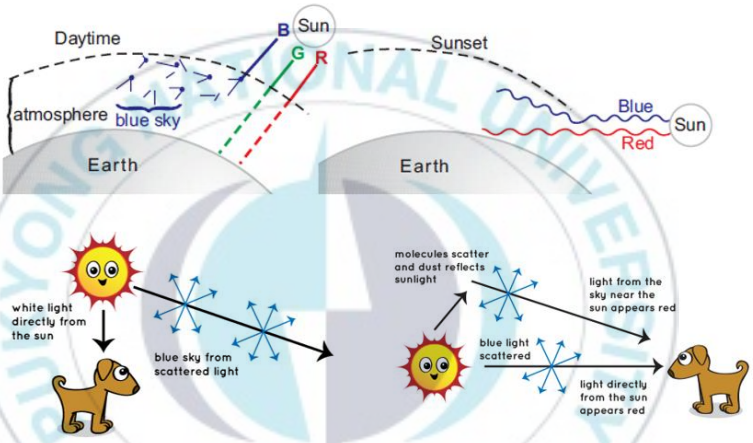


Figure 43: Blue sky at daytime and red sky at sunset explanation

2. Experiment method

a) Gold nanoparticles synthesis

There are two main ways to make AuNPs:

- Top-down approach: From a large piece of material then use mechanical methods (drill, blast, ultrasonic,…) or electrical methods (electromagnetic wave) to reduce the size of the material to nano pieces (Figure 44). This method has one downside is that the equipment for the whole process is costly to achieve nano-sized precision.

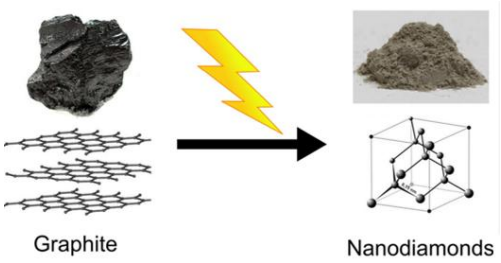


Figure 44: Turn graphite into nanodiamond by breaking up graphite's atomic structure using high-energy sound waves

- Bottom-up approach: Take a molecule that has the right atom then perform the chemical reaction to build up the nanoparticles atom by atom (Figure 45).

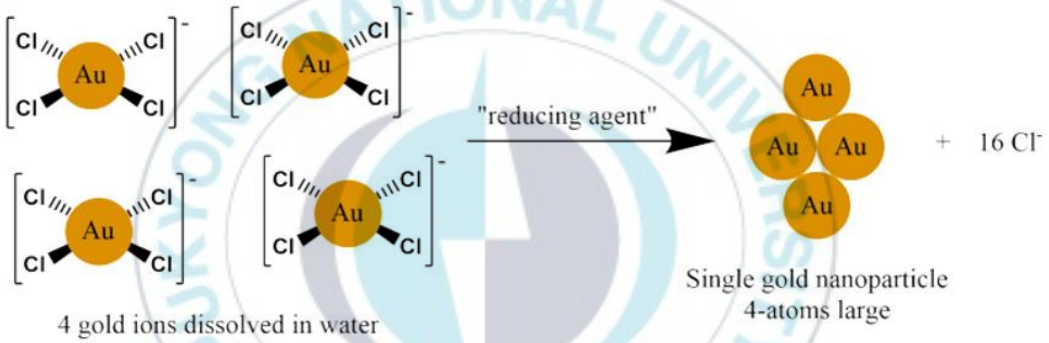


Figure 45: Chemical method to make AuNPs from AuCl

The problem with the bottom-up method is that those gold atoms will continue to grow into bigger lumps of gold. Without any control methods, nano-sized of gold cannot be obtained. The real target is to control gold atoms formation into well-defined nanoparticles, like Figure 46.

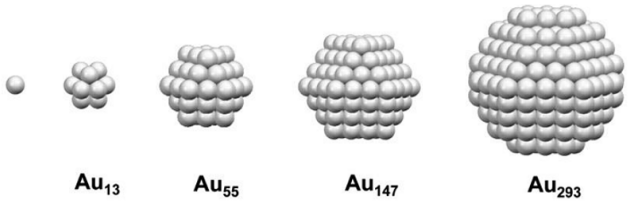


Figure 46: Different size of AuNPs, depends on the number of atoms

Different size of AuNPs will have different color because of light scattering. If AuNPs is so small, then blue light is likely to scatter more, only red light can reflect and reach our eyes. If AuNPs size is big, then blue light is scattered less. That's why we can observe more blue in the solution (Figure 47).

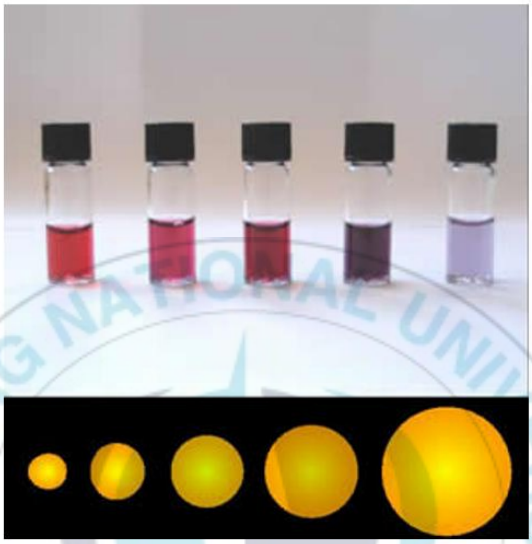


Figure 47: Different size of AuNPs solution has different color

There are a lot of methods to synthesis AuNPs, in this study, the chemical reaction between Sodium Borohydride ($NaBH_4$) and Gold (III) Chloride Hydrate ($HAuCl_4$) was used (Figure 48).

Figure 48: Sodium Borohydride and Gold (III) Chloride Hydrate

$$4HAuCl_4 + 3NaBH_4 = 4Au + 6H_2 + 3NaCl + 3BCl_3$$

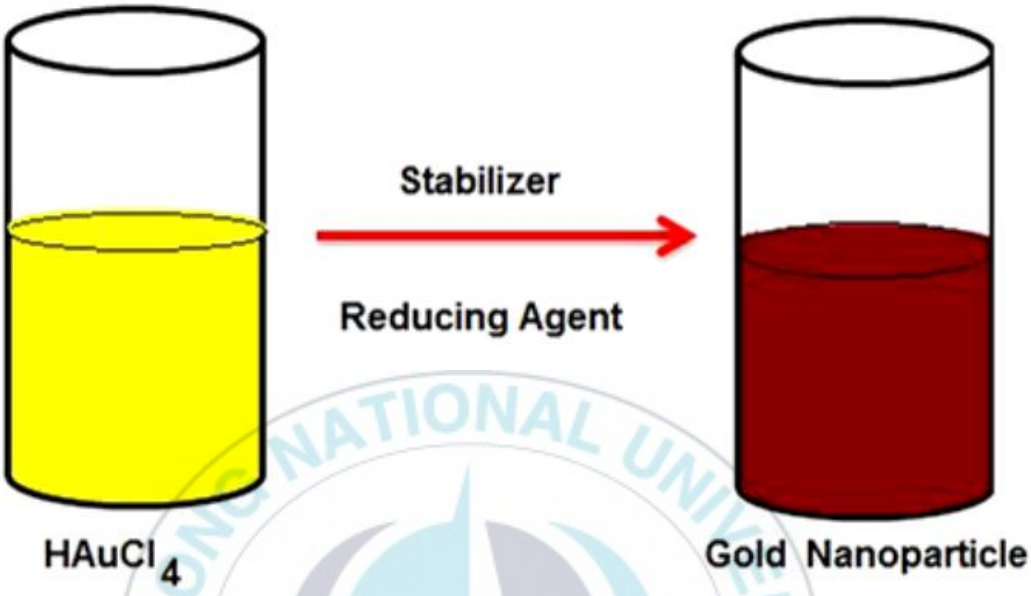


Figure 49: From gold salt to gold nanoparticles

Step 1: Prepare the solution:

$HAuCl_4$: Molar Mass = 339.785 g/mol

$NaBH_4$: Molar Mass = 37.83 g/mol

Step 1: Dissolve $HAuCl_4$ in deionized water and shaken properly to mix solution. Then boil it with magnetic stirring machine

Step 2: Add $NABH_4$ solution drop by drop with continuous stirring, this is acting at reduce and stabilizer agent. After a while, the color of $HAuCl_4$ solution changed from pale yellow to more red.

Step 3: Check the solution by UV-absorption spectra machine, different size of AuNPs will return different wavelength absorbance rate.

If the requirement is not met, add more reduce agent and repeat step 2

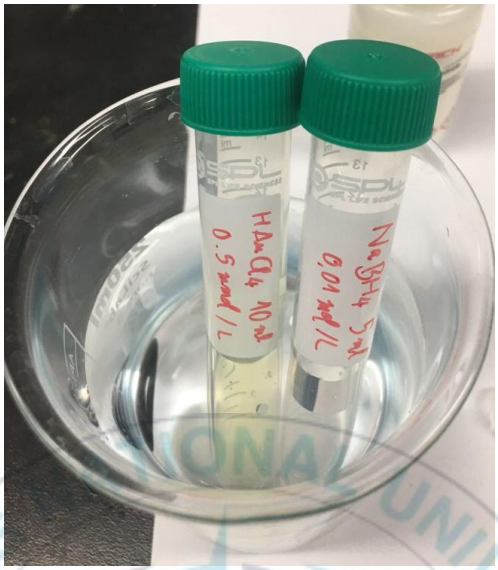


Figure 50: Preparing the solution

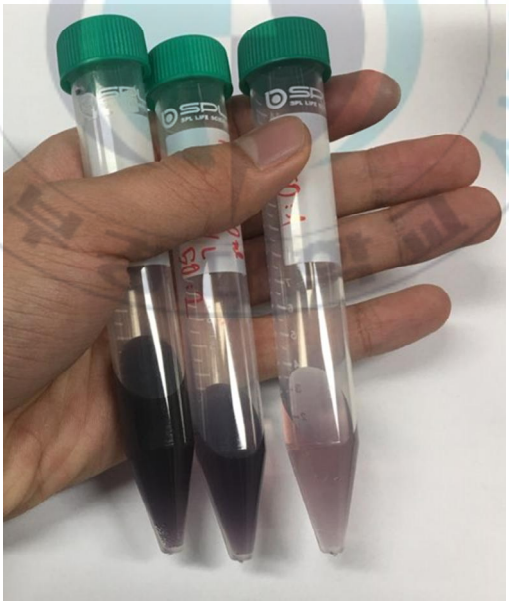


Figure 51: Gold nanoparticles at different concentration

b) Experiment set up

Breast cancer cell MDA-MB-231 was used in this study (Figure 52).

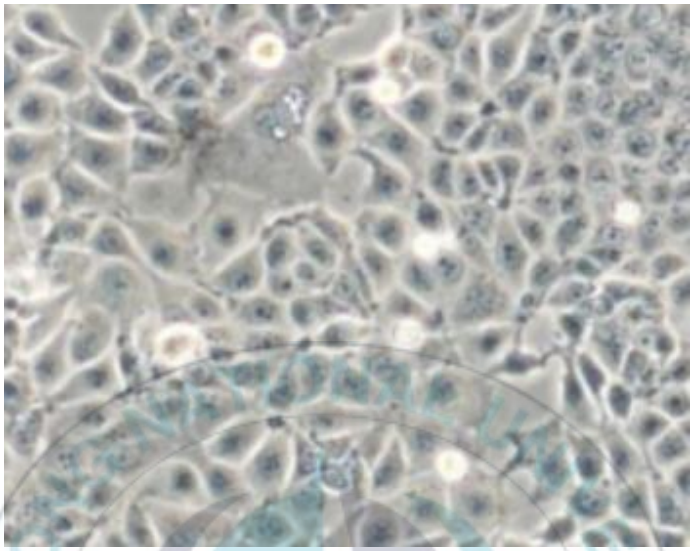


Figure 52: Breast cancer cell MDA-MB-231 image under microscopy

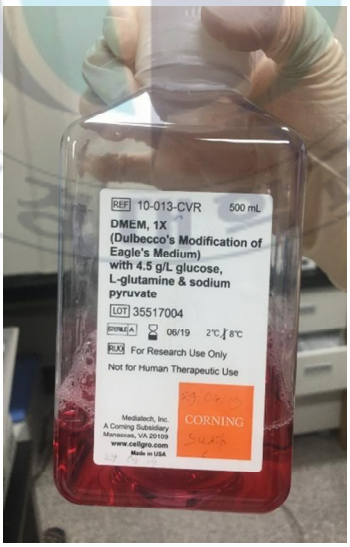


Figure 53: DMEM (Dulbecco's Modified Eagle Medium): Food for cells, which is the concentration of glucose at proper pH and read-to-use liquid

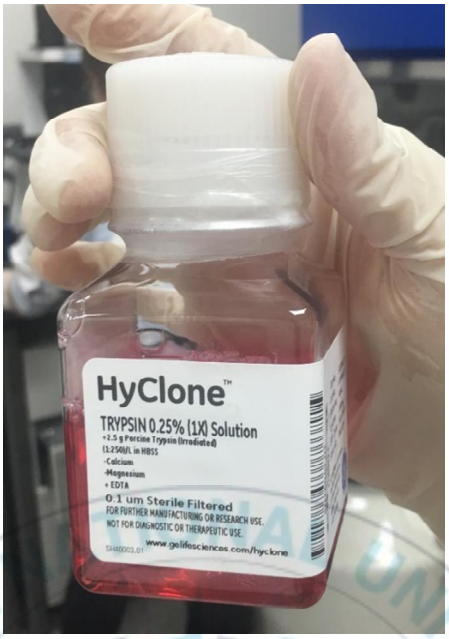


Figure 54: Trypsin: Cells were routinely passaged using trypsin. Normal water cannot pass all the cell from one plate to another.

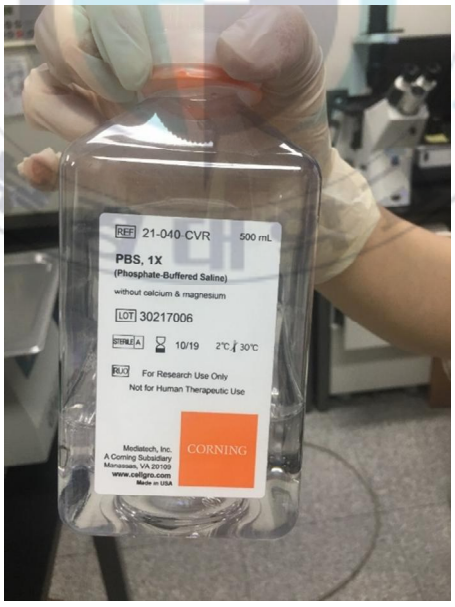


Figure 55: Phosphate: Clean the plate after experiment. Distilled water cannot clean the plate because cancer cells still stick to the plate.

After the uptake between cancer cell and gold nanoparticles. A simple testing device was designed by Solidworks program and then print by 3D printer. The principle of this device is a cuvette holder and isolator to environment light source.

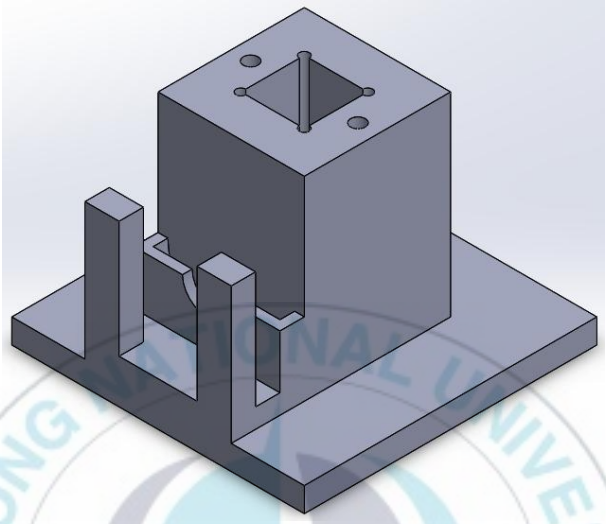


Figure 56: Cuvette holder design by Solidworks

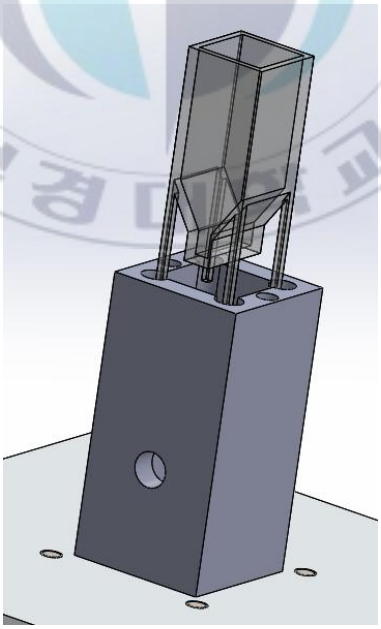


Figure 57: Cuvette is inserted into the cuvette holder

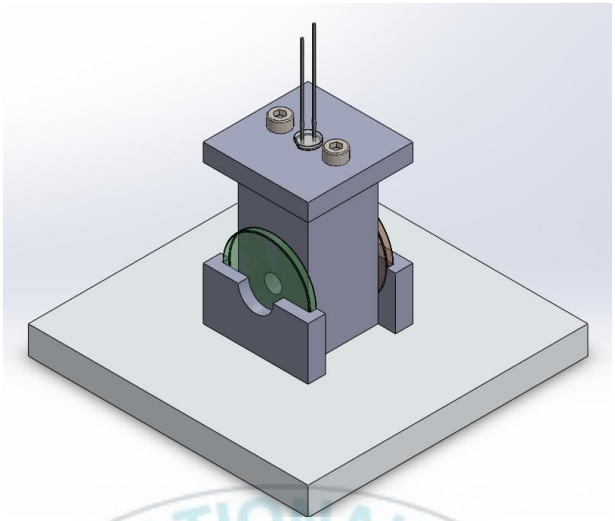


Figure 58: Cuvette holder has been sealed from environment light, the only light source is the White LED on the top

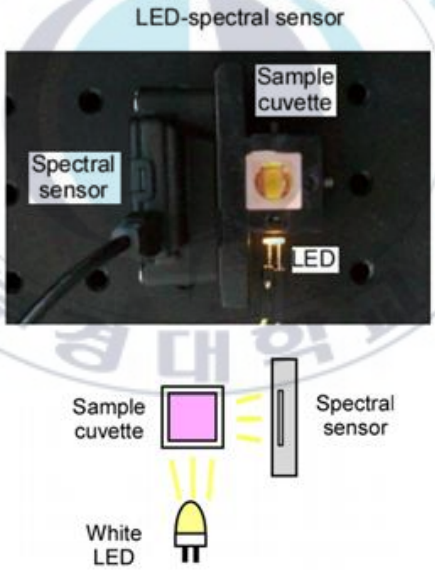


Figure 59: Spectral sensor is used to sense the light intensity come out of cuvette holder

Figure 60: VIS range Spectral sensor AS7262

Experiment will be executed with deionized water, pure gold nanoparticle and cancer cell uptake gold nanoparticle solution. The spectral sensor can measure the intensity of red light and green light after Rayleigh scattering process. The differences between them can be used to calculate the amount of cells uptake AuNPs according to Beer's law.

References

1. Glibver Vasquez, Yulán Hernández, Yves Coello, *Portable low-cost instrumentation for monitoring Rayleigh scattering from chemical sensors based on metallic nanoparticles*. Scientific Reports, 2018.
2. Yi-Cheun Yeh, *Gold nanoparticles: preparation, properties, and applications in bionanotechnology*. Nanoscale, 2012, 4, 1871.
3. Katsu Toshi Miura., *Scanning acoustic microscopy for characterization of neoplastic and inflammatory lesions of lymph nodes*. Scientific Reports, 2013.
4. Arakawa M., *A method for the design of ultrasonic devices for scanning acoustic microscopy using impulsive signals*. Ultrasonics, 2018.
5. Yulán Hernández, *Highly sensitive ratiometric quantification of cyanide in water with gold nanoparticle via Resonance Rayleigh Scattering*. Talanta, 2017.
6. T. Kundu., *Properties of copper film characterized by scanning acoustic microscope*. Biophysical Journal, 2000.
7. Katsu Toshi Miura., *A scanning acoustic microscope discriminates cancer cells in fluid*. Scientific Report, 2015.
8. William D. O'Brien, JR., *Single-element transducers*. Radio graphics, 1993. 13:P947-957.
9. Cheol-Kyou Lee, Yoshinibu Murakami,. *Improvement of the precision of ultrasonic microscope for biological tissue using automatic extraction of the reference signals*. IEEJ Transactions on fundamentals and materials, 2005. 125(2): p145-152.
10. Yoshifumi Saijo, Esmeraldo Santos Filho,. *Ultrasonic tissue characterization of atherosclerosis by a speed-of-sound microscanning system*. IEEE trans Ultrason Ferroelectr Freq Control, 2007. 54(8): 1571-7.
11. Thomas, Luthi (2013) *Non-destructive evaluation methods*

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