CHAPTER 4

EXPERIMENT

Previous chapter shown the simulations part, this chapter represents the part of the experiments. The first experiment is design, construction, and test of the deceleration lens and the next experiments are designing and construction of the deceleration lens system for ion bombardment of DNA, designing and construction of the deceleration lens system for beam energy measurement, installation of the deceleration lens systems, measurement of ion beam energy, DNA preparation, bombardment of DNA, and finally gel electrophoresis for analyzing plasmid DNA forms.

4.1 Designing, construction, and test of the deceleration lens

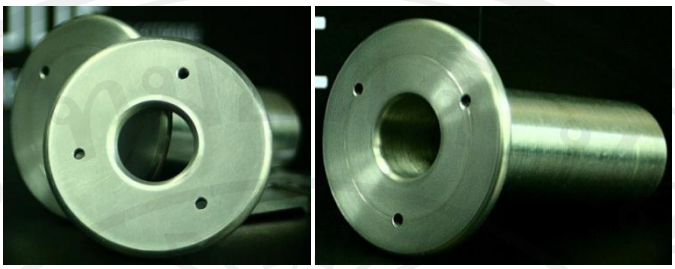
In the previous chapter, the deceleration lens was simulated by SIMION program version 8.0. In this section, designing, construction, and test of the deceleration lens are described.

The deceleration lens consists of 6 Al-electrodes, which are made from aluminium (Al). Each electrode is cylindrical shape with an outer diameter of 60 mm, an inner diameter of 20 mm and a thickness of 5 mm. Only the last electrode is a cylindrical plate connected with a tube in a length of 85 mm. The setup of the

electrode plates and the deceleration lens are shown in Figure 4.1. Teflon insulator is used to separate each electrode at 25 mm apart, except that between the third and the fourth electrodes at 30 mm. The Teflon rod has a diameter of 10 mm, positioned between electrodes at a diameter of 40 mm. The dielectric breakdown of Teflon is 60 MV/m. An Al cylindrical cover protects the deceleration lens and is connected with the lens by 4 legs of Teflon insulator. The cover is in a diameter of 100 mm and a length of 250 mm and has a 10-mm hole at center for ion beam to pass through the deceleration lens.

The deceleration lens was tested for a breakdown of Teflon and air insulators under atmospheric pressure. A gigaohm meter and high-voltage power supply of 30 kV provided potentials to the electrode (voltage limitations of the gigaohm meter is 10 kV and power supply is 30 kV). The procedure of the testing breakdown of the deceleration lens is as following. The gigaohm meter or the power supply gives a high voltage to the first electrode and a ground to the second electrode. The gigaohm meter or the power supply increases the voltage until breakdown which give the limitation of the deceleration lens about 30 kV. Then, each consecutive electrode pair and between the sixth with the cover was tested. A diagram of the breakdown test is shown in Figure 4.2.

The deceleration lens was separated in 2 groups according to their roles, the focusing lens and the deceleration lens part. The former consisted of the first 3 electrodes, which can be called an einzel lens for focusing ion beams before entering the deceleration part. The first and third electrodes were grounded and the second



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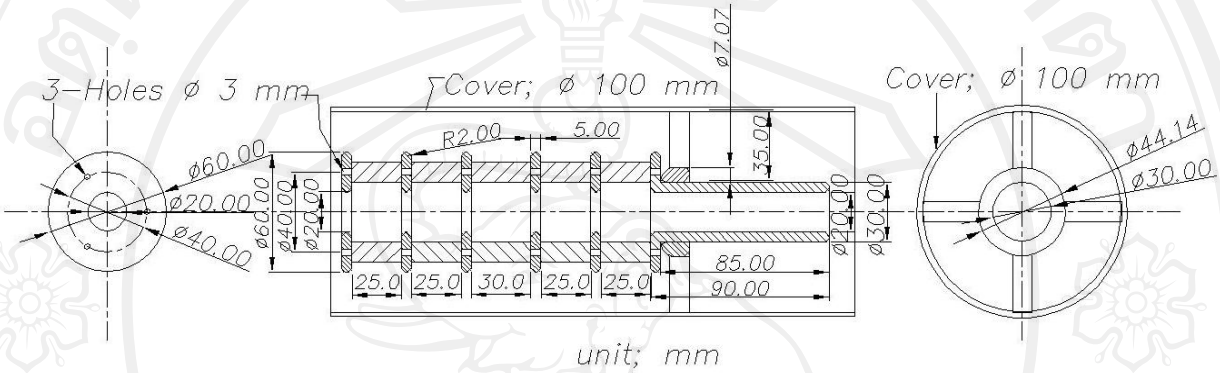

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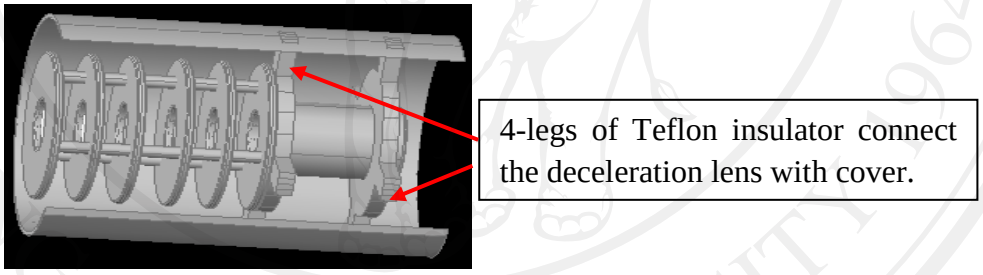

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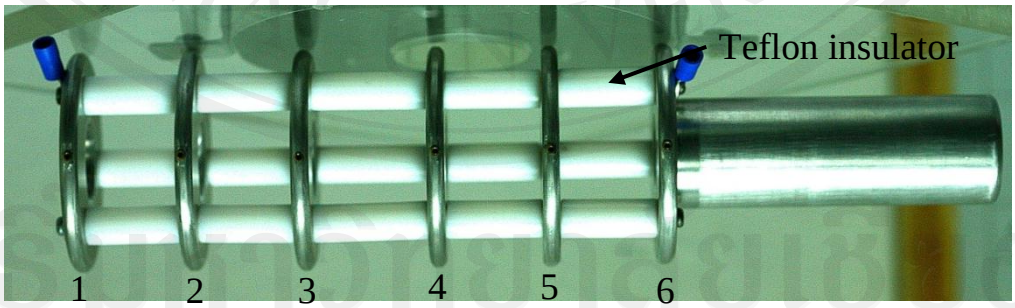


(d)

Figure 4.1. The constructed deceleration lens. (a) Photograph of Al-electrodes. (b) Schematic and (c) drawing of the deceleration lens. (d) Photograph of the finished six deceleration lens.

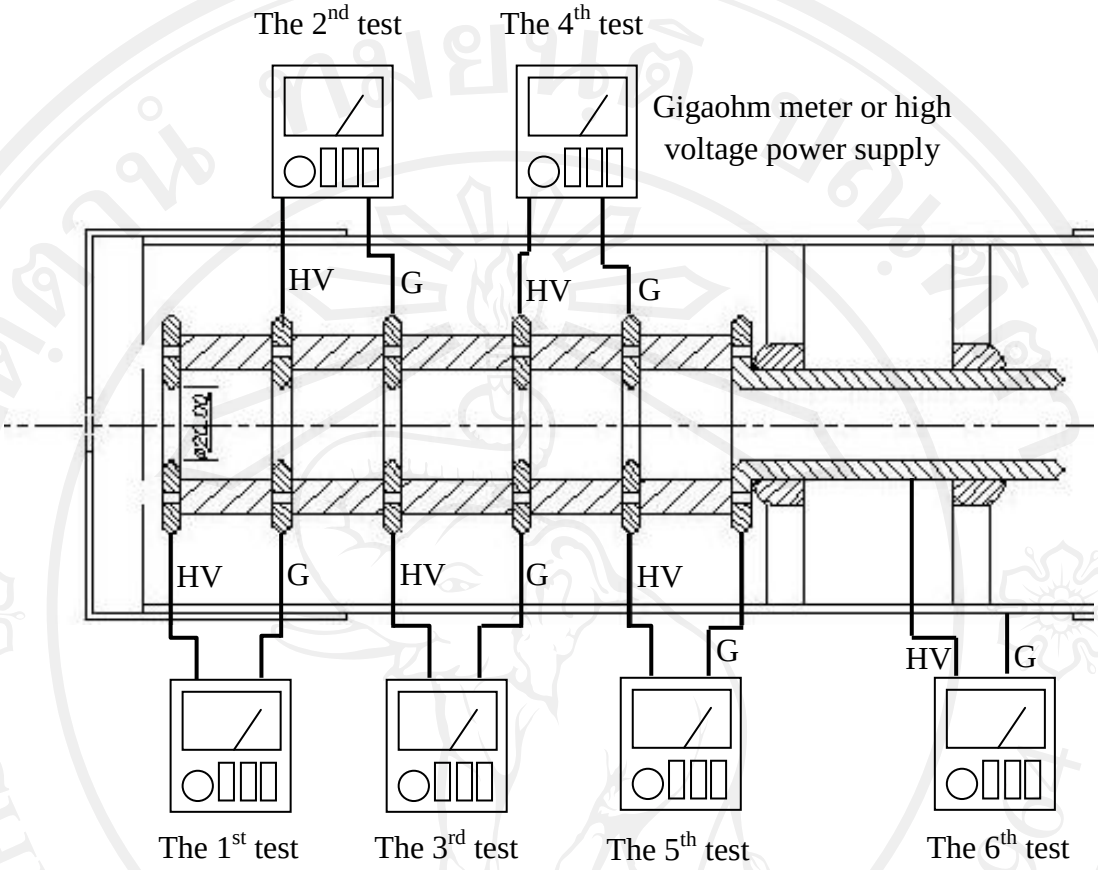


Figure 4.2. Schematic of the breakdown test of the deceleration lens. HV: high volt. G: ground potential.

electrode is high voltage. The deceleration part consists of the last 3 electrodes for reducing ion beam energy while the sixth electrode plays a role in making the exiting ion beam parallel. The deceleration lens was supported by 2 high voltage power supplies. The first one was for the Einzel lens and the other for the deceleration part (the 15 kV Spellman SL150 power supply for the Einzel lens and the 30 kV EH series Glassman high voltage power supply for deceleration part). Both supplied potentials to the deceleration lens electrodes. The schematic of this setup is shown in Figure 4.3.

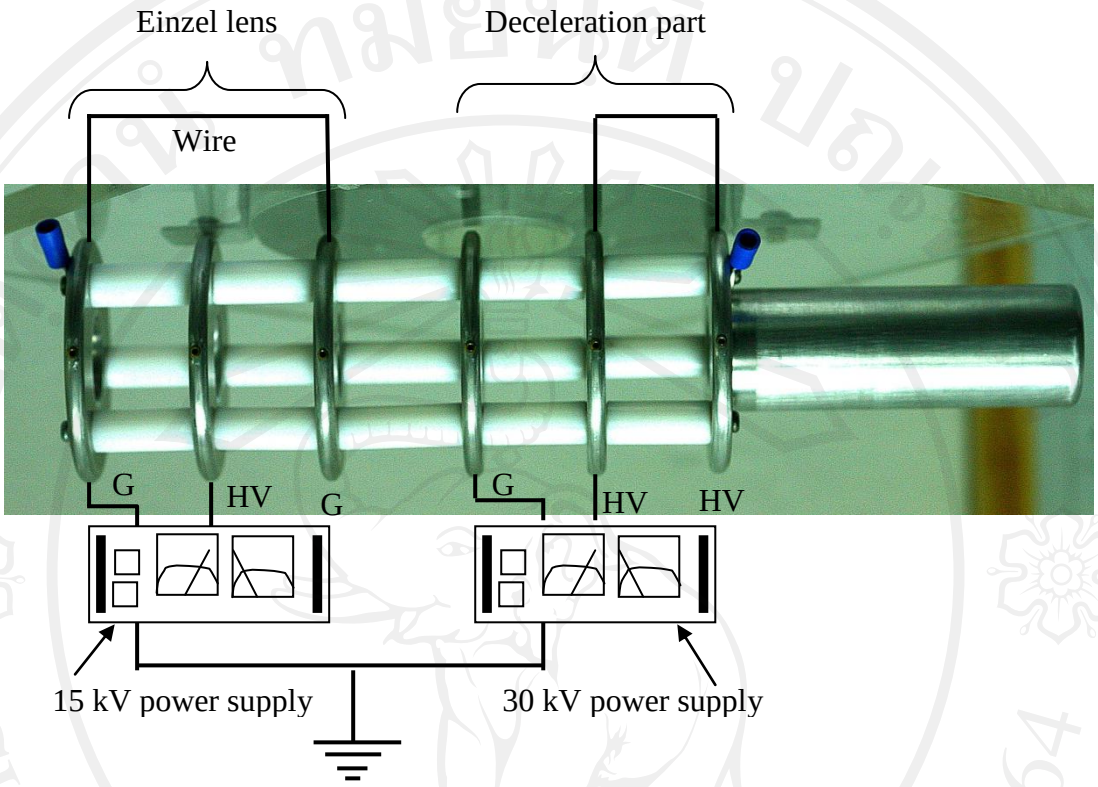


Figure 4.3. Six deceleration lens supported by two power supplies, 15 and 30 kV.

4.2 Designing and construction of the deceleration lens system for ion bombardment of DNA

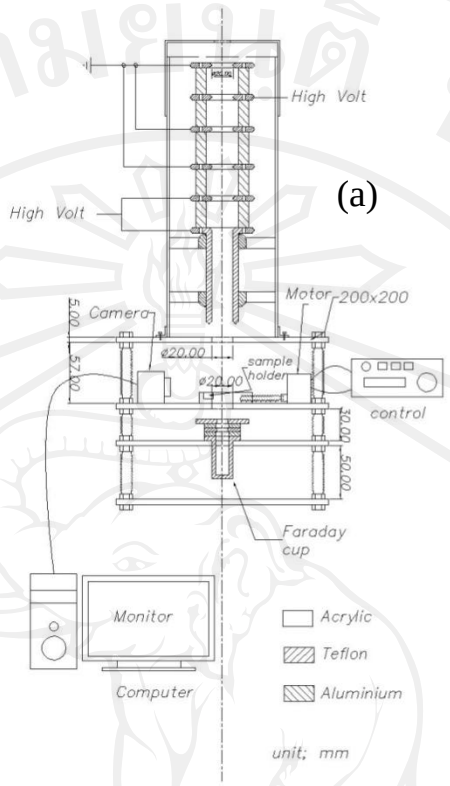
The deceleration lens system for bombardment DNA consists of 3 parts, as shown in Figure 4.4. The first part is the deceleration lens with the cover and cables for high voltage supplying to electrodes. This part having 6 electrodes was explained in the previous section. The second part, under the deceleration lens, is a sample stage placing under the deceleration lens for DNA sample holder. This stage is equipped with a webcam-camera for monitoring sample and stepping-motor to translate the sample holder in one-direction. Camera shows real-time operation at the monitor and

the stepping-motor is controlled by a controller. The third part, being under the sample stage, is a Faraday cup with a secondary electron suppressor for ion beam current measurement. All stages are supported by a 20 x 20 cm^2 acrylic plates.

4.3 Designing and construction of the deceleration lens system for beam energy measurement

The deceleration lens system for measuring ion beam energy consists of 3 parts as shown in Figure 4.5. The first part is the deceleration lens with the cover and cables. The second part, under the deceleration lens, is used as electrostatic plates for bending ion beam. The plates are square of 30 x 30 mm^2 and covered by Teflon insulator for reducing the edge focusing effect due to a non-uniform electrostatic field. The thickness of the plates is 2 mm and the gap between the two plates is 20 mm.

The third part is designed for detecting the ion beam current. This part consists of a copper rod (1 mm x 40 mm) with base being moved by a stepping motor. A stepping motor with a controller, a webcam-camera showing the copper rod moving, connected to a monitor, a vernier caliper for measuring position of ion beam bending by the electrostatic plates, and a multi-meter connected to the copper rod for measuring the beam current. The distance between the plates and the copper rod is 40 mm (x' parameter in Figure 2.8). The equipment is constructed in order to measure the ion beam energy and demonstrating the simulation described in Section 3.3.



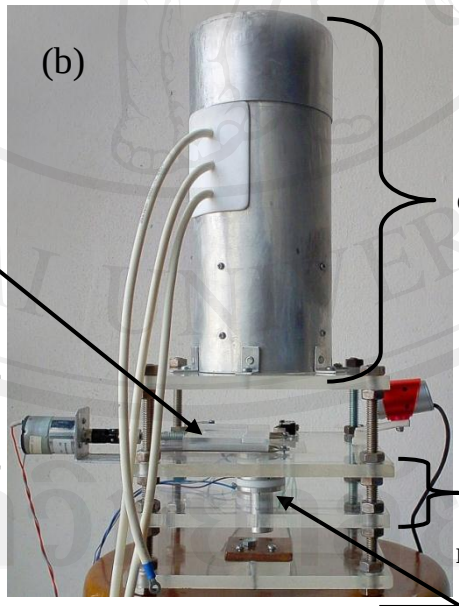
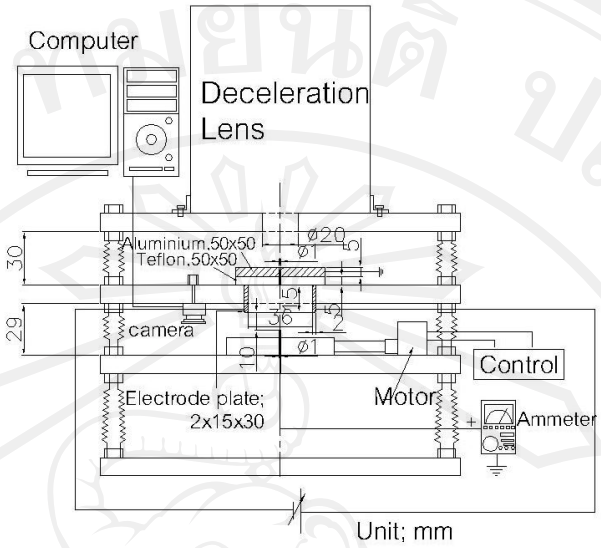


Figure 4.4. The entire deceleration lens system. (a) schematic drawing and (b) photograph of the system for DNA bombardment.



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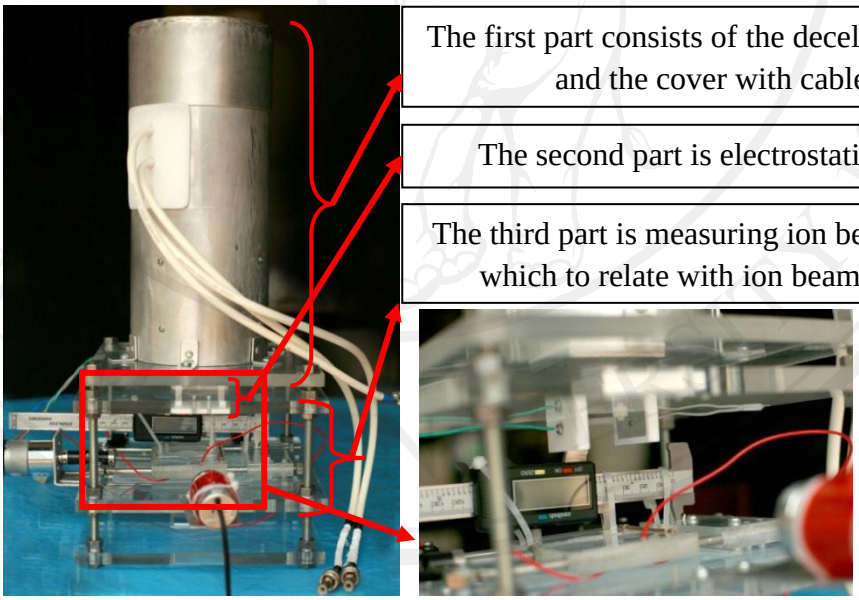


(b)

Figure 4.5. The deceleration lens system for ion beam energy measurement. (a) Schematic drawing and (b) photograph of deceleration lens system for measurement of ion beam energy. The inset in (b) at the right hand side shows enlargement details of the ion energy measurement part.

4.4 Installation of the deceleration lens systems

The deceleration lens systems, for DNA bombardment and beam energy measurement are installed in the beam line of the 30-kV vertical bioengineering ion implanter (CMU3) in the big chamber, as shown in Figure 4.6. There are feedthroughes to connect between controllers outside the big chamber with the deceleration lens systems inside the big chamber. Vacuum system is supported by rotary and turbo pumps, where the rotary pump can get a pressure in order of 10^{-2} torr and the turbo pump can reach a pressure in order of 10^{-6} torr. The deceleration lens systems are operated under a pressure about 10^{-4} torr. The stepping-motor is controlled by a controller box outside the big chamber which can adjust the velocity of the stepping-motor. The webcam-camera is connected to the computer for monitoring the sample and position from the verniar caliper.

4.5 Measurement of ion beam energy

The basic idea is to use an electrostatic field to bend the beam, depending on the ion beam energy. The theory was described in Section 2.6 already. This section describes procedures for measurement of ion beam energy. The system for measurement of ion beam energy is shown in Figure 4.7.

The procedures of measurement of ion beam energy are shown step by step.

4.5.1 Prepare the 30-kV vertical bioengineering ion beam line for measurement of ion beam energy and bombardment of naked DNA will be described

in the appendix A. In the first step, the deceleration lens and the electrostatic plates are not turned on.

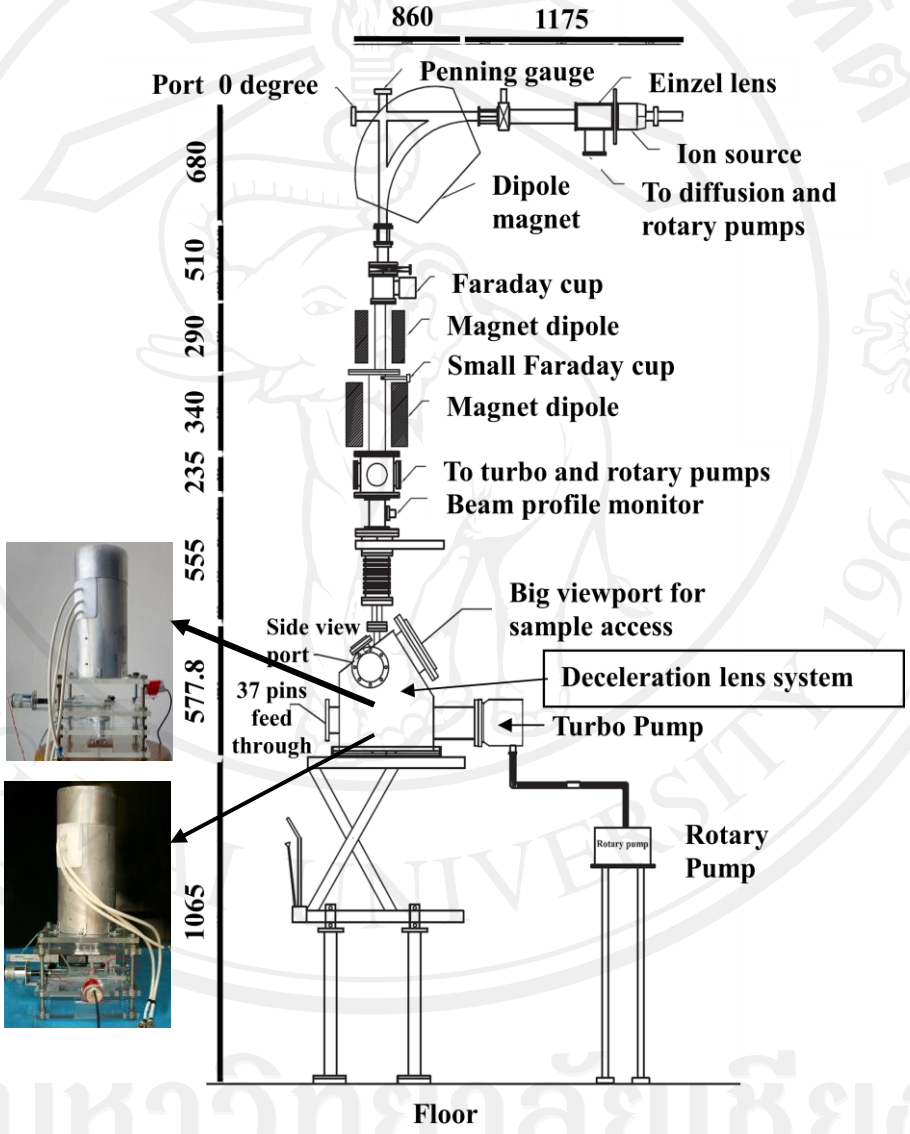


Figure 4.6. The deceleration lens systems installed in the beam line of the 30-kV vertical bioengineering ion implanter (CMU3) in the big chamber.

4.5.2 Open the gate value at the small chamber in order to connect the vacuum system of the big chamber (pressures in the small chamber about $4x10^{-5}$ torr and in the big chamber about 4×10^{-4} torr). In the second step, the deceleration lens is opened but the electrostatic plates are not turned on.

4.5.3 Move the copper rod for detecting the ion beam current driven by the stepping-motor, and the position of the copper rod is displayed by the webcam-camera.

4.5.4 The copper rod is moved step by step in millimeter by millimeter and the ion beam current as a function of the position is recorded.

4.5.5 Summarize the data of the ion beam current as a function of the position.

4.5.6 The data is plotted by the Microsoft Office Excel 2007 program, and the beam profile along the beam bending position can be known.

4.5.7 Power supplies (15 kV and 30 kV) supply voltages to the electrodes of the deceleration lens and the voltages given to the electrodes follow the simulation by SIMION program version 8.0.

4.5.8 Repeat steps 3) to 6) to obtain the beam profile for the case of the deceleration lens turned on but the electrostatic plates are turned off.

4.5.9 Turn on the electrostatic plates for bending the ion beam. Power supplies (15 kV and 30 kV) give voltages to the electrodes of the deceleration lens and the voltages given the electrodes follow the simulation by SIMION program version 8.0. The DC power supply gives voltages to the electrostatic plates. The power supplies are shown in Figure 4.8.

4.5.10 Repeat steps 3) to 6) to get the beam profile for the case of both the deceleration lens and the electrostatic plates turned on.

4.5.11 Finally, obtain 3 beam profiles.

4.5.12 Beam profiles are compared so that we can know the distance of an ion beam bending by an electrostatic field, as shown for example in Figure 4.9.

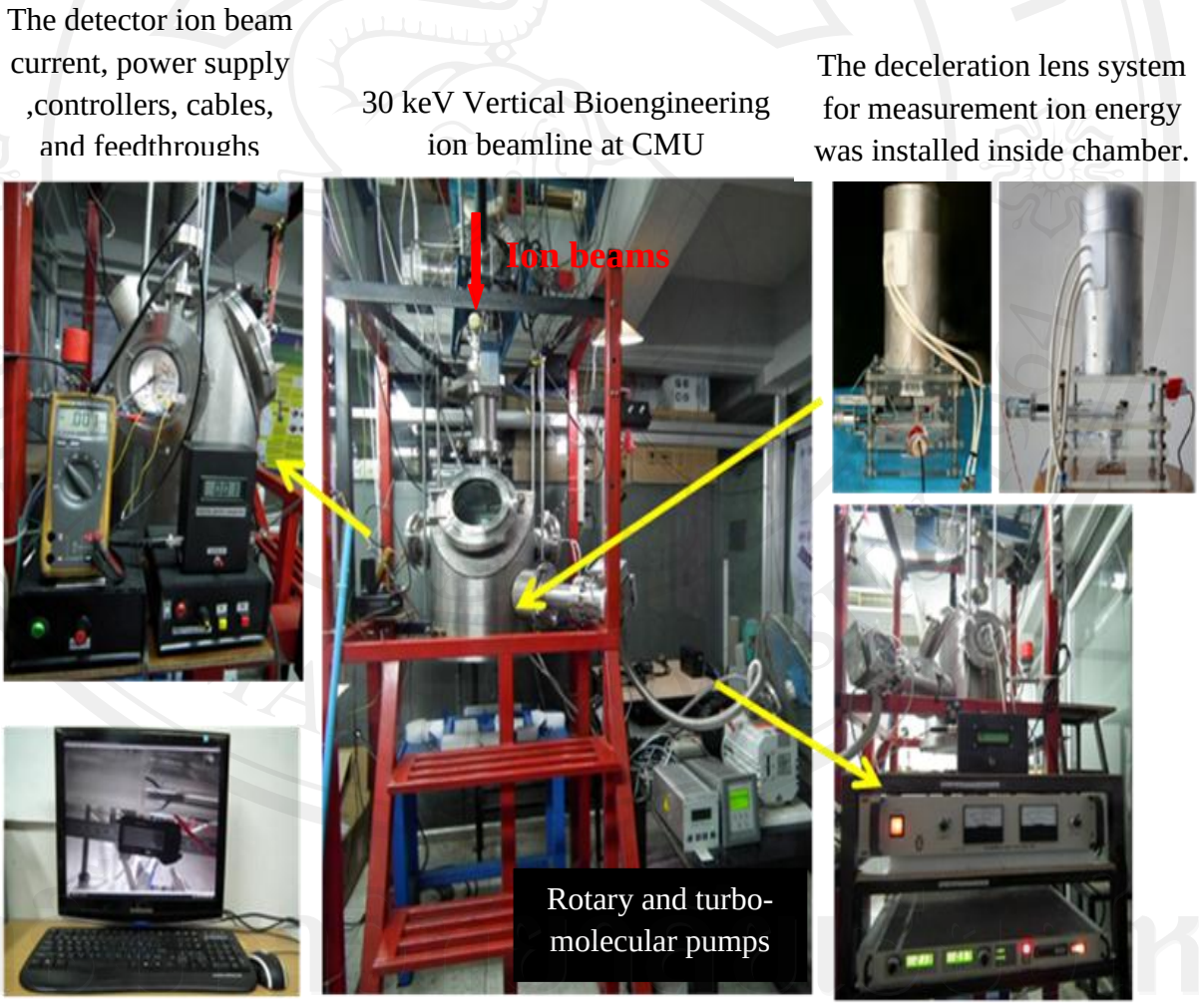


Figure 4.7. The system for measurement of ion beam energy.

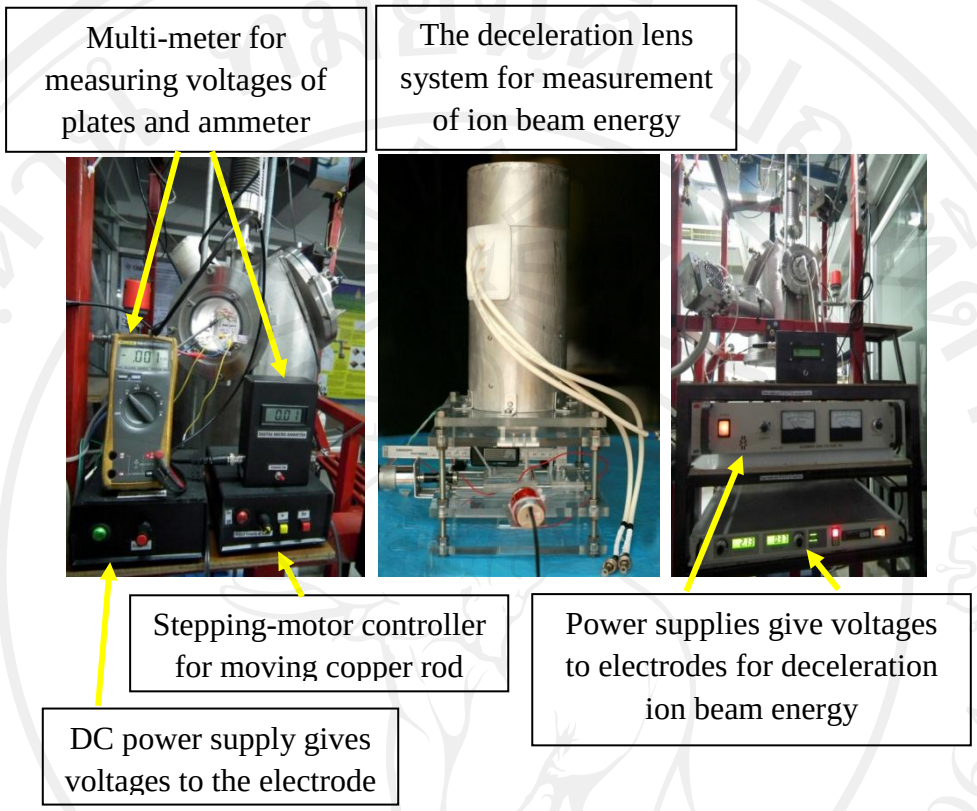


Figure 4.8. The equipment used for ion beam energy measurement.

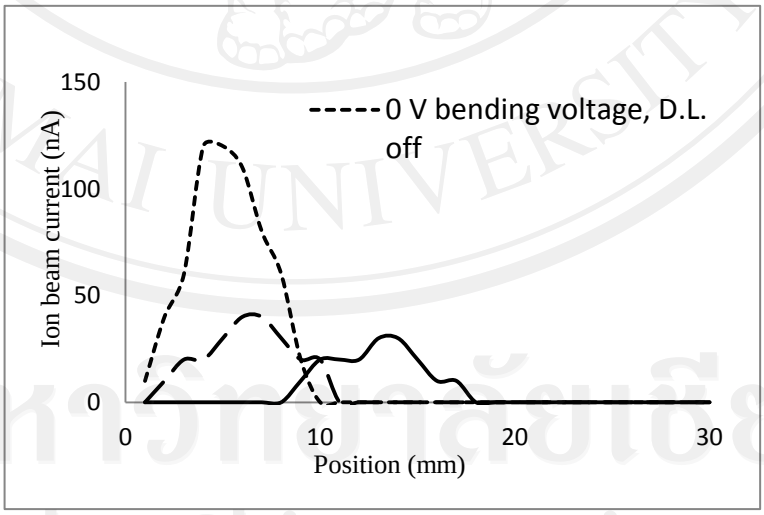


Figure 4.9. An example of measured ion beam profile along the beam bending distance for each case. D.L.: deceleration lens. The beam profile center is taken as the beam position for calculation.

4.6 DNA preparation

The former work concentrated at the designing, construction, installation of the deceleration lens system, and measurements the ultra-low ion beam energy on order to confirm the simulations. This work, the deceleration lens system for DNA bombardment was used then the naked DNA samples were prepared. The procedures of preparation naked DNA sample are shown step by step.

4.6.1 Plasmid DNA is extracted by genomic DNA extraction kit®(Minikits and Cell lysis buffer for Bacteria).

4.6.2 To measure the quantity of naked DNA by spectrophotometer, for to know DNA concentration.

4.6.3 The naked DNA is dissolved in sterile, de-ionized water resulting in a plasmid concentration 200 µg/µl.

4.6.4 The naked DNA was dropped in the holes of glass holder, which each hole has the naked DNA in 1 µl. The glass sample has 4 holes, the first 3 holes for bombarded and the last hole in order to compare is vacuum control for compared effect between DNA bombarded and non-bombarded at vacuum pressure.

4.6.5 The naked DNA was dried in laminar flow for 30 minutes.

4.7 Bombardment of DNA

The deceleration lens is tested and the ion beam energy is measured already. The deceleration lens is used in the biophysics field, in which this thesis emphasizes ion bombardment of DNA at ultra-low ion beam energy. The deceleration lens system

for bombardment of DNA is shown in Figure 4.10. The procedures for bombardment DNA are described as following.

4.7.1 Prepare the 30-kV vertical bioengineering ion beam line (details will be explained in the appendix A).

4.7.2 All gage vales are opened in order to connect vacuum systems (ion source, small chamber, and big chamber parts).

4.7.3 Power supplies give voltages to the electrodes of the deceleration lens (The voltages follow the simulation and the measured ion beam energy in the previous experiment).

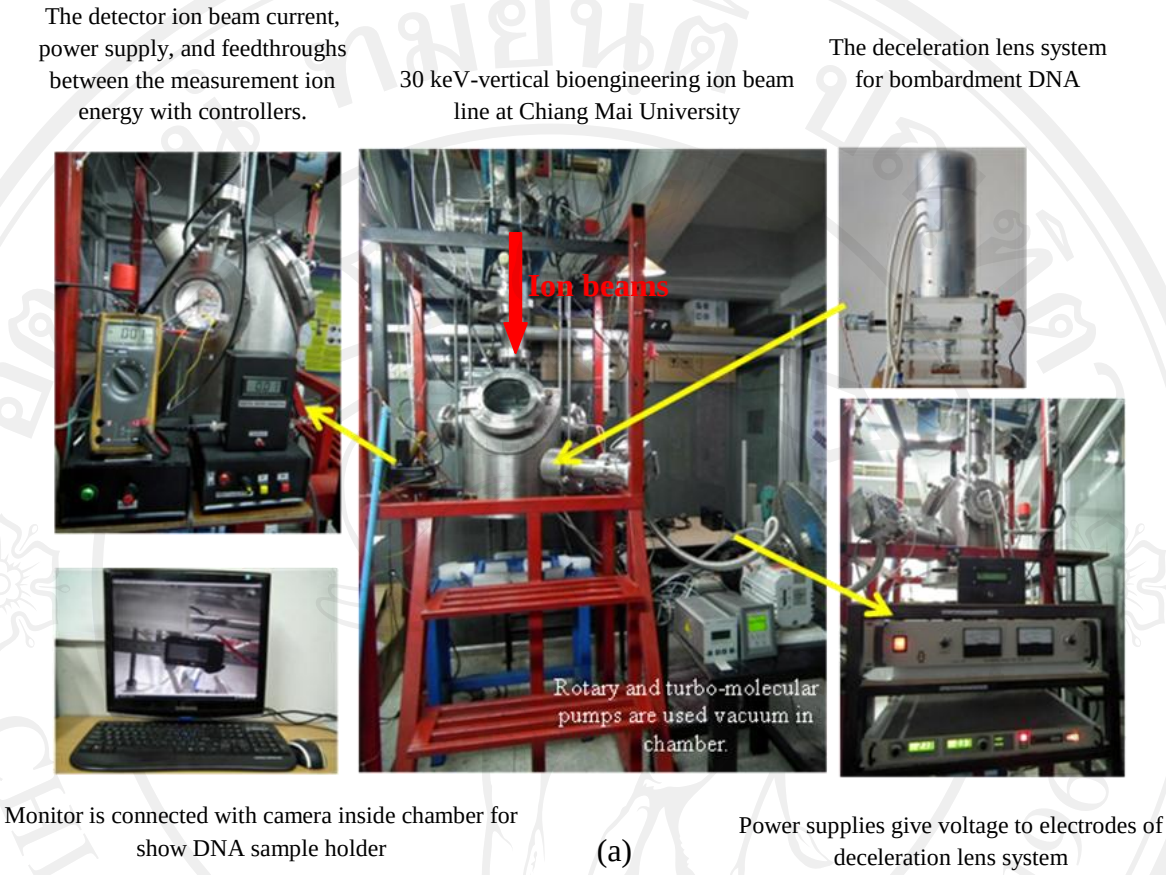
4.7.4 Ion beam current is measured by the Faraday cup.

4.7.5 Naked DNA samples are bombarded; the time for bombardment of DNA depends on the calculated fluence and the ion beam current following equation (2.89).

4.7.6 The naked DNAs were bombarded by nitrogen (N_2^+) and argon (Ar^+) ions at the fluence of 1 x 10^{15} ions/cm^2. The ion beam energies were 242, 304, 407, and 510 eV for argon ion beam whereas the ion beam energy of nitrogen was 64 eV.

4.8 Gel electrophoresis for analyzing plasmid DNA forms

The DNA was bombarded by argon or nitrogen ion beam with an ion fluence of 1x10^{15} ions/cm^2 and varied energies of 242, 304, 407, and 510 eV for argon ion



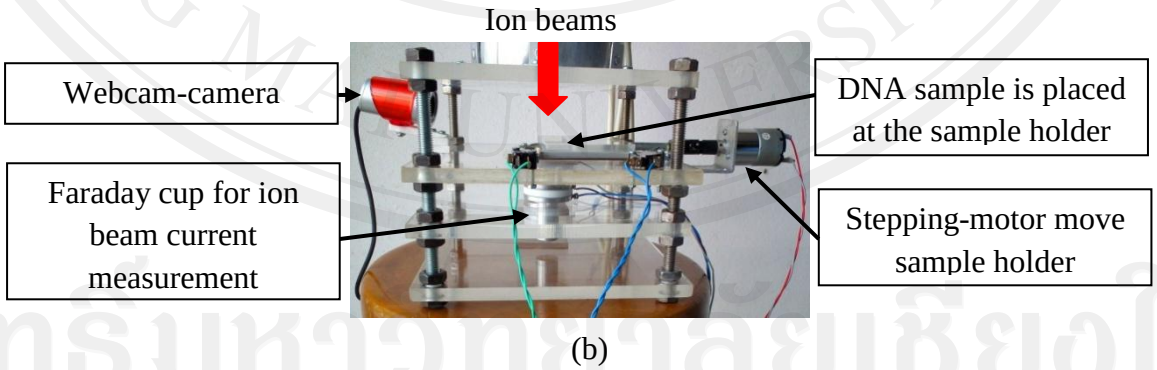


Figure 4.10. Illustration of decelerated ion beam bombardment of naked DNA. (a) System for bombardment of DNA. (b) Sample holder and Faraday cup.

beam but for nitrogen ion (N_2^+) beam the fluence of $1x10^{15}$ ions/cm^2 and ion beam energy of 64 eV. For each condition, DNA samples of natural control and vacuum control were also prepared for comparing the effect of ion beam on DNA conformation change. Each treatment was made for 3 replications.

Gel electrophoresis is a simple technique for analyzing DNA forms. The procedures of analyzing DNA forms is shown as following.

4.8.1 After bombardment of DNA by deceleration lens system, the DNA sample was added with 10-µl de-ionized water and loaded with dye.

4.8.2 Agarose gel was prepared at 1.4% and it dissolve in TBE 0.5x at 100 ml.

4.8.3 The DNA sample was run gel electrophoresis in agarose gel at 50 volt for 50 minutes.

4.8.4 The DNA was stained with ethydium bromide and observed for DNA bands under UV light.

4.8.5 Photos were taken and input to the OriginPro8 program to calculate the light intensity of each band. The results of the natural control, the vacuum control and each treated sample were compared.