Photo-thermally enhanced temperature gradient gel electrophoresis for DNA separation
A Dissertation Proposal Presented

By

Sichao Hou

To

The Department of Chemical Engineering

In partial fulfillment of the requirements
For the degree of

Doctor of Philosophy

In the field of

Chemical Engineering

Northeastern University
Boston, Massachusetts

July 10th, 2018
ACKNOWLEDGEMENT

First, I would like to thank my advisor, Prof. Ming Su for sharing his great idea to me as my PhD project. His enlightening advice and patient guidance gives me great motivation to learn more and dig deeper in my field. I learnt how to manage my time efficiently to have the highest yield and how to write scientifically in a logical way.

I would like to thank the members of my committee for their patience and support in overcoming numerous obstacles I have been facing through my research. I would like to thank my fellow students and postdocs in our department for their feedback and cooperation. In addition I would like to express my gratitude to Rob Eagan for helping me fabricate different types of devices and parts.

I would like to thank my friends for accepting nothing less than excellence from me. Last but not the least, I would like to thank NSF and NIH for funding.
ABSTRACT

A great challenge of genomics and proteomics is the repeatable and reproducible separation of DNA and proteins with high resolution. Gel electrophoresis is irreplaceably applied for separation and isolation of macromolecules, including DNA, RNA and protein, by providing diffusion resistance to molecules of different size and shape. The separation capability of gel electrophoresis is relatively low for long DNA segment limited by the modest voltage employed for the separation, even for high voltage capillary electrophoresis system. On the other hand, temperature that can affect all physicochemical properties of solution, gel and macromolecules, plays a significant role in gel electrophoresis. Although uncontrolled temperature variation in electrophoresis is considered pestiferous to separation, leading to low reproducibility of separation and thermal degradation of sensitive analytes, a controlled variation in temperature can be beneficial to separation. Temperature has a strong influence on the diffusion coefficient, which determines migration rate in gel electrophoresis. Temperature, at the meantime, affects the structural and mechanical properties of gel such as pore size, gelation rate and elastic modulus, among which the pore size has a significant impact on diffusion.

This project describes a photothermal method to enhance gel electrophoretic separation of DNAs. A photothermal temperature gradient is created within an agarose gel using a digital micromirror device (DMD) to dynamically examine the effect of temperature gradient on DNA separation. Temperature gradient between 20-60 °C was established in gel with dynamically controlled patterns. The relation between light brightness and temperature, luminance, and power density was measured for quantitative analysis. While the effect of gel thickness can be ignored in heat transfer.
Then the phase behavior of agarose gel was observed by in-situ measuring the light transmission and the hysteresis loop is obtained. Nucleation and growth mechanism was used to describe the gelling and melting process. Pore size of the agarose gel was then calculated based on the transmission measurement and found it increases with the temperature and decreases with the concentration. The temperature effect on mechanical strength and thermal conductivity will facilitate the operation of photothermal enhanced electrophoresis.

Finally, the enhancement of separation capability has been determined. Both double-strand DNA and single-strand DNA marker have been used to verify the photothermal enhanced electrophoresis. Compared to static light control, dynamic light control creates a more uniform temperature gradient along the migration of DNA and the separation capability is higher. Effect of electrophoretic voltage and gel concentration on DNA migration mobility has been discussed and found high voltage and low concentration lead to a higher migration mobility. With the help of temperature gradient, longer DNA has a better enhancement ratio under certain voltage and concentration. The enhancement effect is especially significant for higher gel concentration.

Compared to other temperature gradient electrophoresis, remote temperature generation and detection eliminate thermal lag and potential contamination, providing a clean and precise method for DNA separation. The flexible light control can be used to selectively separate DNA in certain length region, even DNA enrichment by inversely applying the temperature gradient. This work casts a new light on the role of temperature in tuning separation capability of gel electrophoresis for DNA fragments. Given the conciseness and flexibility, the DMD controlled photothermal temperature gradient can be a powerful way to enhance separation capability of a given gel in electrophoresis.
# TABLE OF CONTENTS

ACKNOWLEDGEMENT ........................................................................................................... ii

ABSTRACT ............................................................................................................................ ii

LIST OF FIGURES ................................................................................................................ vi

1.0 INTRODUCTION ................................................................................................................ 1

2.0 LITERATURE REVIEW ..................................................................................................... 4

2.1 Gel electrophoresis ........................................................................................................... 4

2.1.1 Classification of electrophoresis .................................................................................. 4

2.1.1 Agarose gel electrophoresis ....................................................................................... 6

2.1.3 Mechanism of electrophoresis ................................................................................... 8

2.1.4 Temperature effect on gel electrophoresis ................................................................. 14

2.2 Digital micromirror device controlled photothermal heating ...................................... 16

2.3 Infrared thermal imaging system .................................................................................. 19

2.3.1 Infrared radiation .................................................................................................... 20

2.3.2 Emissivity ................................................................................................................ 21

2.3.3 IR imaging system .................................................................................................... 23

3.0 EXPERIMENTAL SECTION ............................................................................................ 26

3.1 Synthesis of fluorescence labeled agarose gel ............................................................... 26

3.2 Photothermal system ..................................................................................................... 26

3.3 DNA migration on photothermally enhanced gel electrophoresis ................................ 27

3.4 Characterizations .......................................................................................................... 28

3.4.1 UV-vis light transmission ......................................................................................... 28

3.4.2 Mechanical strength ................................................................................................. 30

3.4.3 Thermal conductivity ............................................................................................... 30

4.0 PHOTOTHERMAL TEMPERATURE GRADIENT BUILDUP ........................................ 32

4.1 Calibration of emissivity of black receptor and agarose gel ........................................ 32

4.2 Temperature control on black receptor ........................................................................ 33

4.3 Temperature control on agarose gel ............................................................................ 35

4.4 Heat transfer along the direction of thickness of the gel ............................................. 38

4.5 Summary ....................................................................................................................... 39

5.0 TEMPERATURE EFFECT ON GEL PROPERTIES .................................................... 40
5.1 Temperature effect on phase transition of agarose gel.................................40
5.2 Temperature effect on gel pore size.............................................................45
5.3 Temperature effect on mechanical and thermal properties.............................48
5.4 Summary ........................................................................................................49

6.0 PHOTOTHERMAL ENHANCED GEL ELECTROPHORESIS FOR DNA
SEPARATION...........................................................................................................50

6.1 DNA migrations in static and dynamic light control.........................................50
6.2 Effect of electrophoresis output voltage on DNA migration..............................54
6.3 Effect of gel concentration on DNA migration................................................57
6.4 Photothermally enhanced electrophoresis for DNA separation.........................60
6.5 DNA enrichment by inversely application of temperature gradient...............61

6.6 Summary ........................................................................................................63

7.0 CONCLUSIONS .............................................................................................65

8.0 REFERENCES .................................................................................................67
LIST OF FIGURES

Figure 1: Mechanism of moving boundary electrophoresis (A), zone electrophoresis (B), isotachophoresis (C) and isoelectric focusing electrophoresis (D) ................................................................. 4

Figure 2. AB repeat unit of agarose polymer: (A) 1,3 linked β-D-galactose; (B) 1,4 linked 3,6-anhydro-α-L-galactose .................................................................................................................. 7

Figure 3. Formation of agarose gel from sol state. ................................................................. 8

Figure 4. DNA double helix in agarose gel electrophoresis. .................................................... 11

Figure 5. Ogston sieving model (A) and reptation model (B) for DNA migration in agarose gel electrophoresis. ................................................................................................................. 13

Figure 6. Complete melting (A) and partial melting (B) of DNA double strands. ................... 14

Figure 7. Molecular structure change of A-T and C-G base pair at high temperature. The hydrogen bonds will partially break up as temperature increases. .............................................. 15

Figure 8. Electromagnetic spectrum with highlighted visible and infrared regions. .............. 18

Figure 9. Components and working mechanism of digital micromirror device. ...................... 19

Figure 10. Emissivity of black body, grey body and selective radiator (A); dependence of emissivity on viewing angle (B), wavelength (C), and temperature (D). 86-87 ................................. 22

Figure 11. Main components of IR camera. ............................................................................. 24

Figure 12. Enhanced gel electrophoretic separation of DNAs in dynamically controlled photothermal temperature gradient. ................................................................. 28

Figure 13. In-situ UV-vis light transmission measurement of agarose gel in gelling process. .... 29

Figure 14. In-situ UV-vis light transmission measurement of agarose gel in melting process. ... 30

Figure 15. Calibration of emissivity on black receptor (A) and agarose gel (B) at different temperature. .................................................................................................................. 33
Figure 16. Infrared images of temperature patterns on black receptor with different distance from the light source (d) and brightness (b). (A) d=12 cm, b=100%; (B) d=12 cm, b=100~50%; (C) d=12 cm, b=50~5%; (D) d=6 cm, b=100%; (E) d=6 cm, b=100~50%; (F) d=6 cm, b=50~5%.

Figure 17. Temperature distribution on agarose gel with different distance from the light source and brightness.

Figure 18. Infrared images of surface temperature gradient on agarose gel with different light brightness and patterns. (A) Brightness=5%; (B) brightness=100%; (C) brightness=5~100%; (D)-(F) multiple temperature gradients generated by varying brightness from 5 to 100% controlled by DMD.

Figure 19. Areas and ranges of temperature gradient on agarose hydrogel (A); dependence of gel temperature on light brightness and luminance (B); temperature increase of ink heated with different light brightness (C); dependence of output power and power density of the light source on the brightness (D).

Figure 20. Temperature gradient spread in the direction of the thickness. Infrared image of agarose gel faced to (A) and back on to (B) the light energy.

Figure 21. UV-vis light transmission of agarose gel with a concentration of 0.5 wt% (A), 1.0 wt% (B), 1.5 wt% (C), and 2.0 wt% (D) at different temperature in the gelling process.

Figure 22. UV-vis light transmission of agarose gel with a concentration of 0.5 wt% (A), 1.0 wt% (B), 1.5 wt% (C), and 2.0 wt% (D) at different temperature in the melting process.

Figure 23. Hysteresis loop of agarose gel in gelling and melting process with a concentration of 0.5 wt% (A), 1.0 wt% (B), 1.5 wt% (C), and 2.0 wt% (D).

Figure 24. Nucleation and growth mechanism in gelling and melting process of agarose gel.
Figure 25. Determination of agarose gel pore size from water turbidity. Absorbance and water turbidity of agarose gel in 700-800 nm wavelength under different temperature (A); determination of wavelength exponent from wavelength dependent water turbidity (B); determination of pore size under different temperature with wavelength exponent (C)................................. 45

Figure 26. Pore size variation in gelling (A) and melting (B) process of agarose gel with different concentration.......................................................................................................................... 47

Figure 27. Temperature dependent mechanical property of agarose gel at different concentration. Stress and strain curve of agarose gel (A); Young’s modulus as a function of gel concentration and temperature (B). ........................................................................................................... 48

Figure 28. Temperature dependent thermal conductivity of agarose gel at different concentration. Infrared images of top view (A) and front view (B) of agarose with concentration of 0.5 (1), 1.0 (2), 1.5 (3), and 2.0 (4); thermal conductivity as a function of agarose concentration (C). ....... 49

Figure 29. Migration of dsDNA in agarose gel electrophoresis without (1) and with (2) temperature gradient for (A) 1 h, (B) 2 h, (C) 3 h and (D) 4 h. ....................................................................................................................... 51

Figure 30. Optical image of hydrogel electrophoresis of ssDNA ladder under static temperature gradient after migration for 1.5 h (A); dependence of migration speed on temperature and DNA length (B). ....................................................................................................................... 52

Figure 31. Optical image of hydrogel electrophoresis of ssDNA ladder under dynamic temperature gradient after migration for 1.5 h (A); dependence of migration rate on DNA length at temperature of 30, 45, and 60 °C (B); comparison of enhancement ratio in static and dynamic temperature gradient (C) ........................................................................................................... 53

Figure 32. DNA migration in 1 wt% agarose gel electrophoresis under the voltage of 50 (A), 80 (B), 100 (C), 120 (D), and 150 mV (E) for 1 h.............................................................................................................................. 54
Figure 33. DNA length dependent electrophoretic mobility in 1 wt% agarose gel electrophoresis under the voltage of 50 (A), 80 (B), 100 (C), 120 (D), and 150 mV (E). .............................................. 55
Figure 34. Output voltage dependent electrophoretic mobility of DNA with length from 200 to 2000 bp under 30 (A), 45 (B) and 60 °C (C). .................................................................................. 56
Figure 35. Temperature dependent intrinsic electrophoretic mobility of DNA fragments with different lengths. .......................................................................................................................................................... 56
Figure 36. DNA migration in agarose gel electrophoresis with concentration of 0.5 (A), 1.0 (B), 1.5 (C), and 2.0 wt% (D) under the voltage 100 mV for 1 h. ................................................................. 57
Figure 37. Concentration dependent electrophoretic mobility of DNA with length from 200 to 2000 bp under 30 (A), 45 (B) and 60 °C (C). .................................................................................. 58
Figure 38. Temperature dependent electrophoretic mobility of DNA with length from 200 to 2000 bp with concentration of 0.5 (A), 1.0 (B), 1.5 (C), and 2.0 wt% (D). .................................................. 58
Figure 39. Enhancement ratio of photothermal enhanced gel electrophoresis under temperature of 30, 45 and 60 °C. ................................................................................................................................................ 59
Figure 40. DMD controlled photothermal electrophoresis for whole range (A) and selected range (B) enhancement in DNA separation; sketch of pore size tunability of DMD controlled photothermal system in gel electrophoresis(C). .................................................................................................. 60
Figure 41. DNA enrichment along y axis using reverse temperature gradient for 0 (A), 10 (B), 20 (C), 30 (D), 45 (E), and 60 min (F). ............................................................................................................ 62
Figure 42. DNA enrichment along x axis using reverse temperature gradient for 0 (A), 30 (B), 45 (C), and 60 min (D). ............................................................................................................ 62
Figure 43. Enrichment factor of DNA fragment along x axis for 60 min. .......................................... 63
1.0 INTRODUCTION

A great challenge of genomics and proteomics is the repeatable and reproducible separation of DNA and proteins with high resolution. Gel electrophoresis has been developed into an irreplaceable method to identify DNA recombination, analyze interaction between proteins and separate nucleic acids. Gel electrophoresis is widely applied for separation and isolation of macromolecules, including DNA, RNA and protein, by providing diffusion resistance to molecules of different size and shape. However, the separation capability of gel electrophoresis is relatively low for long DNA segment limited by the modest voltage employed for the separation, even for high voltage capillary electrophoresis system. On the other hand, temperature that can affect all physicochemical properties of solution, gel and macromolecules, plays a significant role in gel electrophoresis. Although uncontrolled temperature variation in electrophoresis is considered pestiferous to separation, leading to low reproducibility of separation and thermal degradation of sensitive analytes, a controlled variation in temperature can be beneficial to separation. Temperature has a strong influence on the diffusion coefficient, which determines migration rate in gel electrophoresis. Temperature, at the meantime, affects the structural and mechanical properties of gel such as pore size, gelation rate and elastic modulus, among which the pore size has a significant impact on diffusion.

This project describes a photothermal method to control the pore size of agarose, hence enhance gel electrophoretic separation of DNAs. A photothermal temperature gradient is created within an agarose gel using a digital micromirror device (DMD) to dynamically examine the effect of temperature gradient on DNA separation. Temperature gradient between 20-60 °C was established in gel with dynamically controlled patterns. Then the phase behavior of agarose gel was observed by in-situ measuring the light transmission and the hysteresis loop is obtained.
Nucleation and growth mechanism was used to describe the gelling and melting process. Pore size of the agarose gel was then calculated based on the transmission measurement and found it increases with the temperature and decreases with the concentration. Finally, the enhancement of separation capability has been determined. Both double-strand DNA and single-strand DNA marker have been used to verify the photothermal enhanced electrophoresis. Compared to static light control, dynamic light control creates a more uniform temperature gradient along the migration of DNA and the separation capability is higher. With the help of temperature gradient, longer DNA has a better enhancement ratio under certain voltage, especially for higher gel concentration. The aims of this research project are described in details as follow:

**Aim 1: Photothermal temperature gradient buildup.** An ultra-high power (100 W) light source will be used to generate temperature gradient in agarose gel. A digital micromirror device (DMD) will be used to dynamically control the light patterns and the brightness by program. The surface temperature of hydrogel will be recorded by using an infrared camera operated at a wavelength of 9-13 µm and image resolution of 320×240 pixels, and analyzed using FLIR tools+ software. Temperature gradient between 20-60 °C will be established in gel with dynamically controlled patterns. The relation between light brightness and temperature, luminance, and power density will be measured for quantitative analysis. While the effect of gel thickness can be ignored in heat transfer.

**Aim 2: Temperature effect on gel properties.** UV-vis light transmission of agarose gel with different concentration and temperature will be measured in-situ with a self-built UV-vis spectrometer to study the temperature effect on gel properties. Phase behavior and hysteresis loop of agarose gel will be observed by the in-situ UV-vis spectrometer. The gelling and melting process of agarose gel will be described and explained by nucleation and growth mechanism. Pore size of
the agarose gel will be calculated based on the transmission measurement and the relation between temperature and pore size will be obtained. The temperature effect on mechanical strength and thermal conductivity will also be discussed.

**Aim 3: Photothermal enhanced gel electrophoresis for DNA separation.** The gel electrophoresis of dsDNA and ssDNA will be tested at a constant voltage for 1-4 hrs. DsDNA will be extracted from T lymphoblast cells from human (CCRF-CEM, ATCC). SsDNA ladders (200 to 2,000 base pair) will be used to determine migration rate and ratio of enhancement of temperature gradient electrophoresis. The separation bands will be imaged with a fluorescence imaging system under 210 nm ultraviolet excitation. The influence of both static light control and dynamic light control will be studied on temperature gradient generation along the migration of DNA and the separation capability. Effect of electrophoretic voltage and gel concentration on DNA migration mobility will be discussed to find the highest migration mobility. At last, this method will be used for DNA enrichment in two dimension by inversely applying the temperature gradient.

Compared to other temperature gradient electrophoresis, remote temperature generation and detection eliminate thermal lag and potential contamination, providing a clean and precise method for DNA separation. The flexible light control can be used to selectively separate DNA in certain length region, even DNA enrichment by inversely applying the temperature gradient. This work casts a new light on the role of temperature in tuning separation capability of gel electrophoresis for DNA fragments. Given the conciseness and flexibility, the DMD controlled photothermal temperature gradient can be a powerful way to enhance separation capability of a given gel in electrophoresis.
2.0 LITERATURE REVIEW

2.1 Gel electrophoresis

2.1.1 Classification of electrophoresis

Based on the different mechanisms, gel electrophoresis can be divided into four categories: moving boundary electrophoresis, zone electrophoresis, isotachophoresis and isoelectric focusing electrophoresis.\(^1\)\(^-\)\(^3\) Figure 1 shows the sketch of the mechanism of these four types of electrophoresis.

![Mechanism of electrophoresis types](image)

**Figure 1.** Mechanism of moving boundary electrophoresis (A), zone electrophoresis (B), isotachophoresis (C) and isoelectric focusing electrophoresis (D).

**Moving boundary electrophoresis.** This method is the earliest electrophoresis established by Tiselius.\(^4\)\(^-\)\(^6\) As shown in Figure 1A, the to-be-separated sample is placed in one end of the electrophoresis tank and a clear interface can be observed between samples and carrier electrolyte before separation. When the separation starts, charged molecules will move towards the opposite electrode, while the mobility is proportion to its electric charge. Different separation zones is formed during the electrophoresis, but most of them are overlapped to each other. Only the first
separation zone can be completely separated with a clear boundary, which contains the molecules with the fastest migration rate. Each boundary can be observed by optical methods.

**Zone electrophoresis.** As shown in Figure 1B, different samples can be separated into isolated zones in different areas in a uniform buffer solution or carrier electrolyte. The zones of different samples can be observed by using dyes and the peaks of each zone can be readout by densitometer at different excitation wavelength.\(^7\)-\(^8\) The separated zones will be expanded and diluted as the increase of separation time and distance, which has a significant influence on the resolution. Varies types of medium can be used as an addition to weaken the dilution, especially when separated in the gel (agarose gel, polyacrylamide, etc.), the efficiency is remarkably enhanced.\(^9\)-\(^10\) Zone electrophoresis can be subdivided into paper electrophoresis, gel electrophoresis, powder electrophoresis and silk electrophoresis, among which, gel electrophoresis is the most commonly used method for separation of macromolecules, including DNA, RNA and proteins.

**Isotachophoresis.** Samples with varied migration rates are mixed with leading ions and ending ions, which have the highest and lowest migration rate in the electrophoresis. With the external electric field, the samples migrate in the electrophoresis for some time until completely separated.\(^11\)-\(^12\) The separated samples are listed between the leading ions and ending ions in different zones in order of their migrate rates (Figure 1C). Since appropriate electrolyte is absent in the buffer solution to carry the current, the separated zones are connected to each other with clear boundaries, which is called “the self-correction effect”.\(^13\)-\(^14\)

**Isoelectric focusing electrophoresis.** Samples are mixed with amphoteric electrolytes in a buffer solution with a pH gradient. When the sample is in the environment that is lower than its isoelectric point, the sample will be positively charged and move towards the negative electrode;
when the environment is higher than its isoelectric point, the sample will move towards the positive electrode.\textsuperscript{15-16} When the sample migrates to its specific isoelectric point, the net charge will become zero and stay at this point. Finally, samples with different isoelectric points will be concentrated at the same area to form obvious zones with high resolution (Figure 1D).\textsuperscript{17}

\subsection*{2.1.1 Agarose gel electrophoresis}

Agarose is a type of linear polymer extracted from seaweed with an average molecular weight around 120,000. It usually consists of two repeated units: 1,3 linked $\beta$-D-galactose and 1,4 linked 3,6-anhydro-$\alpha$-L-galactose (Figure 2).\textsuperscript{18-19} Based on the source of the agarose, its structure and repeating units may vary, which determines its melting and gelling temperature. Agarose shows a thermal hysteresis phenomenon in the phase transition between liquid and gel, where the melting temperature is usually 40-50 °C lower than the gelling temperature. The gelling and melting temperature may change for different types of agarose. The gelling and melting temperature also depend on the gel concentration, especially when the concentration is lower than 1\%: the higher the concentration is, the higher the gelling and melting point will be.\textsuperscript{20-21} Since the phase transition point is greatly relied on the chemical structure, they can be modified by chemical methods, such as hydroxyethylation.\textsuperscript{22-23} Based on its melting temperature range, different types of agarose can be divided into high-melting-point (HMP) agarose and low-melting-point (LMP) agarose.\textsuperscript{24-26}
Figure 2. AB repeat unit of agarose polymer: (A) 1,3 linked β-D-galactose; (B) 1,4 linked 3,6-anhydro-α-L-galactose.

Agarose gel is suitable for researchers to study the fundamentals of the gelling and melting processes of natural biopolymers, since it is mostly uncharged and simple in composition and electrostatic interactions. For a standard agarose extracted from Gelidium (one type of red algae), the gelling point is about 34-38 °C and the melting point is about 90-95 °C. When the agarose is dispersed into water and raise the temperature to its melting point, it will melt and freely expanded like a wire. When the temperature goes down below the gelling point, it starts to form gel by coil-helix transition. Figure 3 shows the formation of agarose gel from the sol state. Initially, adjacent molecules will entangled to each other to form a double helix structure like DNA. During aging period, the helices will further form filaments with pores among them for macromolecules to path through. Agarose gel can be regarded as an infinite three dimensional network consisted of helices and fibers of polymer. The melting behavior of agarose gel happens at a relatively higher temperature, where the hydrogen bonds between the agarose fibers are weakened and finally broken up. After completely melting, the system becomes transparent as water. The difference between gelling and melting point is called a thermal hysteresis phenomenon, which is believed to be related to the aggregation of helices: in the gelling process, nucleation is the rate-limiting step that controls the kinetics of the gel formation; while it is commonly accepted that the melting process present the true equilibrium process of aggregation.
Figure 3. Formation of agarose gel from sol state.

Agarose gel electrophoresis is one of the ubiquitously used genetic fingerprinting technique in DNA separation, which indicates the genetic diversity of a biological community. Agarose gel has large pore size distribution and a high mechanical strength, even in very low concentration such as below 1 wt%. Usually, the concentration of agarose gel used in electrophoresis ranges from 0.7 to 2.0 wt%, where the gel is strong enough as an anti-convective medium and a suitable range of pore size.\textsuperscript{39-40} For example, the pore size of 1 wt% agarose gel is estimated in the range of 200-500 nm.\textsuperscript{40-42} Although it has a lower resolving capability than polyacrylamide, agarose gel has a greater separation range, which can be used to distinguish DNA fragments from 50-20,000 bp.

2.1.3 Mechanism of electrophoresis

When solid, such as molecules, particles, colloid, etc., contacts with liquid, electrical potential will be generated between them: solid and liquid will bring opposite charges, which is called the electric double layer.\textsuperscript{43-44} Bio-macromolecules are one of the most common types of substance that needs to be separated using electrophoresis, including DNA, RNA, proteins, etc. The surface charges on the bio-macromolecules come from: (1) absorption of certain ions in the solution. Due to the loss of the charged ions in the solution, the liquid will be oppositely charge.
Compared to cations, which have lower degree of hydration, anions are easier to be absorbed by surfaces to become negatively charged; (2) partial electrolysis of the macromolecules. The extent of electrolysis is also affected by the pH of the solution; (3) absorption of dissociated liquid molecules.45-48

Proteins and other polyelectrolytes get charged due to the electrolysis of the functional groups on their surfaces. Proteins have both positive and negative dissociative groups, so called the amphoteric electrolyte, which will be positively charged in acidic environment and negatively charged in alkalescent environment.49-50 The pH value that makes the protein to be neutral is called the isoionic point, which does not necessarily equal to its isoelectric point defined as the stationary point in direct-current electric field. Isoelectric point is usually obtained from experimental measurements.51-52

Because of the existence of the electric double layer, electric potential will be generated between the stern layer and the movable layer, which is called the zeta potential (ζ-potential).53-54 The electro-kinetic phenomenon is the result of the zeta potential. Electrophoresis indicates the phenomenon where charged particles move towards the opposite electric pole in the direct-current electric field. If a small piece of porcelain plate is sealed at the bottom of a U-shaped tube, the liquid level near the cathode will be raised when a voltage is applied. Since the porcelain plate is negatively charged, so the liquid will be correspondingly positively charged and move towards the cathode, which is called the electro-osmosis.55-56 Inversely, an electric potential will be generated if a pressure is applied to push the liquid through the porcelain plate. This potential is called streaming potential.57-58 If the liquid is fixed, a sedimentation potential will be also generated when particles moves, such as the settlement of colloid particles or the raise of bubbles. This process is opposite to electrophoresis.
The migration rate, or called mobility, is one of the most important parameters in electrophoresis, which determines the separation performance, time, voltage, etc. For a spherical particles in the electrophoresis, the driving force by electric field can be expressed:

\[ F = q \cdot E \]  \hspace{1cm} (1)

where \( F \), \( q \), and \( E \) are electrostatic force, quantity of electric charge, and electric field intensity, respectively. At the same time, when the spherical particle moves in the solution, a drag force will be applied on it, which follows the Stokes Law:\n
\[ F' = 6\pi r \eta v \]  \hspace{1cm} (2)

where \( F' \), \( r \), \( \eta \), and \( v \) are resistance force, particle radius, viscosity of the solution, and particle velocity, respectively. In the steady state, the driving force equals the resistance, which means:

\[ q \cdot E = 6\pi r \eta v \]  \hspace{1cm} (3)

By definition, electrophoretic mobility (\( \mu \)) indicates the particle velocity under unit electric field intensity (1 V/cm), thus:

\[ \mu = \frac{v}{E} = \frac{q}{6\pi r \eta} \]  \hspace{1cm} (4)

Usually, the value of the electrophoretic mobility is in the order of \( 10^{-5} \) (cm\(^2\)/V\( \cdot \)s). Under the same environment, electrophoretic mobility is a physicochemical property constant for a certain species.

Among all types of electrophoresis, gel electrophoresis is most widely used and has been developed into an irreplaceably method for separation and isolation of macromolecules by providing diffusion resistance to molecules of different size, shape, and charge.\textsuperscript{60-64} With the electric field applied, charged molecules can be forced to move along or against the field within a
porous medium, usually a hydrogel made of agarose or polyacrylamide. A buffer solution, by providing a reservoir of weak acid and base, is usually used in the gel electrophoresis for both supplying electrolytes and keeping a constant pH, since the charge of DNA and RNA rely on pH.\textsuperscript{65-66} The hydrogel is usually a cross-linked polymer with a tunable porosity and pore size used for different types of targeted molecules with varied size. When the target molecules are small, such as nucleic acids or proteins, acrylamide is the most common composition of the gel, accompanied with a cross-linker to produce polymer networks with different size.\textsuperscript{67-70} When macromolecules are to be separated, including DNA, RNA and large proteins, agarose gel is preferred since its pore size is much larger than that of acrylamide.\textsuperscript{71-74}

![Diagram of DNA double helix in agarose gel electrophoresis]

**Figure 4. DNA double helix in agarose gel electrophoresis.**

The mechanism of DNA migration in gel electrophoresis is explained below.\textsuperscript{75-77} Figure 4 shows the charge of DNA and different layers in gel electrophoresis. First, DNA is negatively charged. The base pairs inside are positively charged while the phosphate backbones outside are negatively charged. Although DNA itself is electrically neutral, but more negative charges are exposed to the environment to attract positive charges. Around DNA, there are two layers of
charges, one is called condensed counterion layer, where lots of positive charges are gathered around DNA; the other one is called diffuse counterion layer, where positive charges are moving towards DNA so the density of positive charges would be a little bit higher than negative ones. The charge density of DNA is affected by many parameters, like dielectric constant, Boltzmann temperature, and also the structure of DNA itself, as illustrated in Eq. 5:

\[
\xi = \frac{q^2}{4\pi\varepsilon_m k_B T_b}
\]  

where \( \xi \), \( q \), \( \varepsilon_m \), \( k_B \), and \( T_b \) are charge density parameter, electric charge, dielectric constant, Boltzmann constant, and Boltzmann temperature, respectively. The negatively charged DNA can migrate towards the positive electrode if an electric field is applied in gel electrophoresis. This picture shows a typical DNA ladders in the agarose gel electrophoresis.

Multiple models have been buildup to explain the DNA migration in agarose gel. First one is the Ogston model (Figure 5A), which regards the agarose gel as a sieve.\textsuperscript{78-80} A random coil DNA will be treated as a small sphere and moves through the interconnected pores. The mobility will degrade exponentially when the size of DNA becomes larger and larger. The second model is the reptation model (Figure 5B).\textsuperscript{81-84} This model treats long DNA fragments as a snake moving through the pores and the mobility is inversely proportional to the molecular weight or length if the gel concentration and electric field are kept the same. In both cases, the larger molecules is more likely to collide with the matrix to reduce their mobility, and therefore, the molecules can be separated for their different sizes through this method.
Figure 5. Ogston sieving model (A) and reptation model (B) for DNA migration in agarose gel electrophoresis.

Despite the widely use of the agarose gel electrophoresis in macromolecule separation, it has many limitations. The first and most fatal limitation is that the separation capability of gel electrophoresis is relatively low for long DNA segment, which is limited by the modest voltage employed for the separation.\textsuperscript{85-88} The low resolving capability stops the application of agarose gel electrophoresis in precise separation of long DNA fragments. Related to the low separation capability, the precision of the separation is also low, especially for large DNA.\textsuperscript{89} The other problem is that during the electrophoresis, heat will be generated by the application of electric field, which may lead to uncontrolled temperature increase, even the melting of agarose gel. Furthermore, the electrophoresis separates different molecules based on the difference of their size and charge, for molecules with the same size and charge, it is difficult or even impossible to be separated by the gel electrophoresis. All the above drawbacks limit the application of agarose gel electrophoresis in certain area where precise separation is required.
2.1.4 Temperature effect on gel electrophoresis

![Figure 6. Complete melting (A) and partial melting (B) of DNA double strands.](image)

There are many factors that could affect the electrophoresis, such as gel types, ion strength, pH, viscosity, buffer concentration, voltage, temperature, etc.\textsuperscript{90-92} Among them, temperature has a strong influence on all the physicochemical properties of solution, gel and macromolecules, plays a significant role in gel electrophoresis. Although uncontrolled temperature variation in electrophoresis is considered pestiferous to separation, leading to low reproducibility of separation, thermal degradation of sensitive analytes, and heat related dispersion, a controlled variation in temperature can be beneficial to separation.\textsuperscript{93} Temperature has a strong influence on the diffusion coefficient, which determines migration rate in gel electrophoresis.\textsuperscript{71, 94-96} At a higher temperature, the bonds between the base pairs will break and the double strand DNA will be uncoiled, which is called DNA melting. The melting temperature varies for different types of DNAs but basically it ranges from 75-85 °C. The melting process doesn’t happen suddenly at the melting temperature but gradually when temperature raises. Below the melting temperature, DNA slowly and partially melts where some of the hydrogen bonds between the base pairs start to break (Figure 5). Figure 6 shows the molecular structures of two base pairs: A-T and C-G. The bond energy for each
hydrogen bond is different. In average, the bond energy between CG pair (-21.34 kcal/mol) is lower than that of AT pair (-14.74 kcal/mol), which means CG are more stable. When the hydrogen bonds break, it will generate more negative charges, which will affect their mobility in the electrophoresis system.

Figure 7. Molecular structure change of A-T and C-G base pair at high temperature. The hydrogen bonds will partially break up as temperature increases.

People use the above property to create the temperature gradient gel electrophoresis. By providing a temperature gradient with a resistive heater in one side of the buffer solution, the mobility of DNA will show difference at different temperatures. For the DNA with the same length, if one sequence includes more AT pairs, it tends to move faster than the one has more CG pairs. Thus this method can be used to separate DNAs of the same length but different sequence, which is called a sequence dependent, size independent method. People have been used this technique
to discover mutations in a DNA or a genome. A variant of TGGE with a transverse temperature gradient was build up to test the temperature effect on DNA mobility.\textsuperscript{100-103} In both cases, an abrupt change in migration rate was observed due to structural change of DNAs above the critical temperature, where the double helix was partially uncoiled between base pairs to create electrostatic charges, but the effect of temperature on the physical properties of hydrogel itself or DNA diffusion were not considered.\textsuperscript{36, 104-105} TGGE has been commercialized, in which an array of resistive heaters was used to generate temperature gradient under gel.

However, this method is not good enough since this system provides a fixed heating position and power limited by the resistive heating method, and the temperature gradient cannot be tuned at will to satisfy specific separation need, which limits its application in other areas. Moreover, the overall separation capability of gel electrophoresis is low especially for longer DNA fragments. So a more tunable system has been considered to be used to enhance the separation of DNA with different length. Temperature, at the meantime, affects the structural and mechanical properties of gel such as pore size, gelation rate and elastic modulus, among which the pore size has a significant impact on diffusion.\textsuperscript{42, 79, 106} It remains unknown whether a controlled temperature field could have a broader impact on DNA separation beyond strand dissociation, and whether this effect could be used to enhance the separation of DNA and other macromolecules.

\textbf{2.2 Digital micromirror device controlled photothermal heating}

Photothermal heating includes different types of techniques based on the conversion of optical energy into thermal energy, usually presented as heat. Numerous types of materials, including solids, liquids, and gases, can absorb the optical energy and eventually convert it into heat. When the optical energy is absorbed by the atoms or molecules, they will be excited to higher energy levels; they lose their excitation energy by non-radiative relaxation pathways, such as
molecular vibration and collision, to generate heating effect in the material. This is the origin of the photothermal effect.

The energy density of light depends on its wavelength: light with shorter wavelength or higher frequency has a higher photon energy, which can be expressed by Planck-Einstein equation shown in Eq. 6:

$$E = h \nu = \frac{hc}{\lambda}$$

where $E$, $h$, $\nu$, $c$ and $\lambda$ are photon energy, Planck constant, frequency, light speed, and wavelength, respectively. When the wavelength is too short (such as x-ray), the energy density of the light is much higher than the gaps between the energy levels of the molecule or atom, which cannot be absorbed to generate heat effect. In the photothermal science, the wavelength of light source ranges from infrared to near ultraviolet based on the optical properties of the targeted heating materials (Figure 8).\textsuperscript{107-109} Near infrared (NIR) light is widely used in the biomedical field as photothermal therapy since the light with a wavelength from 650 to 1350 nm has a maximum penetration depth in human tissue, which is called “NIR window”.\textsuperscript{110-111} Within this wavelength region, most of the photons will be scattered in the light-tissue interaction. The light scattering increases the distance photon can travel through tissue, which also increases the probability of being absorbed. Visible and near ultraviolet light is widely used as a light source to provide heat in physical and chemical processes, such as initiating photocatalytic reactions and tuning physical states.\textsuperscript{112-113}
Figure 8. Electromagnetic spectrum with highlighted visible and infrared regions.

The photothermal heating pattern (or area) can be controlled by a digital micromirror device (DMD).\textsuperscript{114-115} A schematic image of DMD structure is shown in Figure 9. The DMD chip plays a role like an array of reflective micro-mirrors made of aluminum, which can be tuned with a tilted angle of either +10° (“on”) or -10° (“off”) by two bias electrodes. Light can be reflected into the projection lens only when the mirrors are in the state of +10°. When the angle is -10°, the area (or pixel) related to the micromirror becomes dark since the illuminated light will not be reflected into the projection lens. Instead, the light will be collected by a light absorber. The changes of angle in each micromirror is controlled separately by programs from the computer to generate certain image on the screen. The controlled pattern can be used to selectively heat up the areas where the pixel is “on” when a high power light source is matched with the DMD chip.
Figure 9. Components and working mechanism of digital micromirror device.

DMD assisted photothermal heating has its own strengths compared with contact heating: (1) the heating area can be accurately controlled by the illuminated area without affecting the adjacent area; (2) the photothermal heating does not require complicated mechanical and electrical structure to achieve accuracy control; (3) possible thermal lag is eliminated due to the time needed for heat transfer in the resistance heating. The above advantages enable a flexible and controllable heating method for applications in different fields.

2.3 Infrared thermal imaging system

Infrared imaging, or called thermography, detects radiation in infrared range of the electromagnetic spectrum and present images based on the radiation. According to the wavelength to be detected, infrared imaging devices are divided into three categories: short-wavelength infrared (1.4-3 μm), mid-wavelength infrared (3-8 μm) and long-wavelength infrared (8-14 μm). Thermography is an excellent visualized technique that can be applied in a variety of fields in
science, industry, and even military, including condition monitoring, building diagnose, medical imaging, night vision and targeting, etc.

2.3.1 Infrared radiation

On the basis of fundamental physics, any object emits thermal radiation at any temperature higher than absolute zero.\textsuperscript{116-117} The maximum radiant power emitted by the object depends only on its temperature, which is why it is called thermal radiation. In physics, a hypothetical perfect emitter of thermal radiation is called blackbody, which absorbs every incident radiation with any wavelength and from any direction. The energy of thermal radiation emitted by a blackbody under the given temperature and wavelength is defined to be the maximum in all direction. As perfect absorbers and emitters, blackbodies are treated as standard in radiometry. The wavelength dependent total radiant power ($M_\lambda$) of a blackbody at given temperature can be expressed in Planck’s law:

$$M_\lambda(T)d\lambda = \frac{2\pihc^2}{\lambda^5} \frac{1}{\exp\left(\frac{hc}{\lambda kT}\right) - 1}d\lambda$$  \hspace{1cm} \text{(7)}$$

where $\lambda$, $c$, $T$, $h$ and $k$ are wavelength, speed of light, absolute temperature, Planck constant and Boltzmann constant, respectively. The wavelength of maximum radiation power is derived by letting the derivative to be 0 ($dM_\lambda(T)/(d\lambda) = 0$), which is led to Wien’s displacement law:

$$\lambda_{\text{max}} \cdot T = 2897.8 \mu m \cdot K$$ \hspace{1cm} \text{(8)}$$

For blackbody in the temperature of 210 to 2100K, the maximum emission wavelength falls in the infrared range from 1.4 to 14 $\mu m$, which covers the temperature range in different fields of science, industry and military (except extreme situations such as nucleation reaction). The total radiation power is found by integrating the Planck’s law in the whole wavelength range:
\[ M_\lambda(T) = \int_0^\infty M_\lambda(T) d\lambda = \sigma \cdot T^4 \] (9)

where \( \sigma \) is called the Stefan-Boltzmann constant (5.67×10^{-12} \text{ W/m}^2\cdot\text{K}). This relation is called Stefan-Boltzmann law, which indicates that the total energy produced by a blackbody only depends on temperature.

### 2.3.2 Emissivity

Blackbodies are idealized hypothesis. No object in real can emit the same amount of energy as blackbody at any given temperature. The actual emission of thermal radiation can be calibrated by adding a coefficient describing the deviation from the blackbody. This parameter is called emissivity (\( \varepsilon \)) and it describes the ratio of the emitted radiation from a real surface to that emitted by a blackbody under the same temperature. Depending on the quantity used to describe the radiation, four different definitions are used: (1) spectral directional emissivity; (2) spectral hemispherical emissivity; (3) directional total emissivity and (4) forth total hemispherical emissivity.

Based on the dependence of emissivity on wavelength, real objects can be divided into gray body, where emissivity is a constant, and selective emitter with a wavelength dependent emissivity.\(^{118-119}\) For most real application, emissivity of the object can be treated as a constant in thermography, except when dealing with gases or plastic foils, whose emissivity has a strong dependence on wavelength. The emissivity is usually guessed based on Kirchhoff’s law, which indicates that the radiation absorbed by the object equals to the radiation emitted by itself. This law is established based on the assumption that the discussed object is in its thermal equilibrium.
Emissivity is a complex material property that can be affected by many different parameters, including type of material, surface structure, geometry, observation direction, wavelength and temperature (Figure 10). The major parameter is the material itself. In a simplified classification, materials can be divided into metal and nonmetal. Most nonmetallic materials used in thermography, including skin, paper, glass, polymer, etc., can be regarded as gray body and have a relatively high emissivity of above 0.8. On the contrary, metallic materials, especially the polished metals, usually have an emissivity lower than 0.2. Even for the same material, its emissivity can be greatly influenced by the surface structure. For example, a polished metal can reach an emissivity of 0.02, while the value will increase to above 0.8 if the surface is roughened. The possible reason is that based on Kirchhoff’s law, the emissivity is the compensation of reflectivity for an IR non-transparent object. When the surface is highly smoothed, it has a very high reflectivity, thus a low emissivity; when the surface roughness is high, the reduced reflectivity
will compensate to emissivity. The third parameter that affects emissivity is the viewing angle. The emissivity is defined as the ratio of the amount of radiation actually emitted from a surface to that emitted from a blackbody, where the numerator of the ratio depends on viewing angle. Based on large numbers of measurement, it can be approximated that the emissivity changes little when the viewing angle changes in the range of 0 to 45°. But the behavior for larger viewing angles varies for metals and nonmetals. Other parameters, such as geometry, wavelength and temperature, also affect the emissivity and varies from material to material. It is worth mentioning that for temperature influence, if no phase change occurs, the change may not be significant; but when phase changes, the emissivity would change enormously.

To conclude, emissivity is one of the most important properties of the material in thermography, which will directly change the image. The emissivity should be calibrated each time when material properties or measurement condition changes. Calibration of emissivity can be only conducted by specific experiment, since no theoretical relation has been established to predict the emissivity considering all the parameters at the same time.

2.3.3 IR imaging system

An infrared camera is a device that convert infrared radiation with a wavelength in the range of 1.4 to 14 µm into a false color visual image. The image represents the 2D distribution of the thermal radiation emitted by the surface. The main components of an infrared camera includes IR lens system, detectors, video signal generator and display (Figure 11). These components determine the image quality of the infrared camera.
Figure 11. Main components of IR camera.

The detector system is the core of an infrared imaging system and plays a role as transducer, which converts thermal radiation into electrical signals. The quality of the transducer or the detector determines, in great extent, the image quality and the performance of the infrared system. Infrared detectors have to categories: photon detectors and thermal detectors. The former ones initiate a single-step transduction from the thermal radiation into photocurrent by changing the concentration or mobility of the free charge carries within the detector. Thermal detectors include a two-step transduction: first, the thermal radiation is projected onto and absorbed by the detectors to change its temperature; second, the temperature change will generate a variation in the electrical resistance of a bolometer, which will produce an electrical output.

The performance of an infrared imaging system can be evaluated by a series of parameters, including thermal response, temperature accuracy, spatial resolution, frame rate, integration time, etc. Temperature accuracy describes the absolute error of temperature measurement of a blackbody, which is ±2 K or ±2% for most infrared camera. Temperature resolution of the infrared imaging system is determined by the noise equivalent temperature difference (NETD), which evaluate the thermal sensitivity and can be expressed by:
\[ \text{NETD} = \left( \frac{\partial \Phi_{BB}}{\partial T_{BB}} \right)^{-1} \frac{\sqrt{A_D \Delta f}}{D_{\lambda}^*} \]  

where \( \Phi_{BB}, T_{BB}, A_D, \Delta f \) and \( D_{\lambda}^* \) are radiant power, object temperature, responsive area, frequency difference and specific spectral detectivity, respectively. NETD quantifies the minimum temperature difference between a blackbody and its background when the signal intensity is the same as the noise (signal-to-noise ratio=1) for the camera. The smaller of the NETD value, the higher temperature resolution will be for the infrared camera. **Spatial resolution** is determined mainly by the instantaneous field of view (IFOV), which gives the minimum angle for one detector element to sense the radiation from an object. The minimum detectable size of an object at any given distance can be calculated from \textit{object size} = \textit{IFOV}·\textit{distance}. Besides IFOV, the spatial resolution is also affected by the slit response function (SRF), which considers the influence of the diffraction of the optics. **Time resolution** is important for transient thermal analysis and can be characterized with frame rate and integration time. For a static or a slowly moving object, the requirement upon time resolution is low compared to a fast moving object, such as a ball falls from 10 m height.
3.0 EXPERIMENTAL SECTION

3.1 Synthesis of fluorescence labeled agarose gel

All chemicals were obtained from Sigma-Aldrich or Alfa Aesar and used without purification. 0.5-2.0 g agarose powder was added into 1x TAE buffer solution (tris base:acetic acid:EDTA=40:20:1) at ~90 °C (gel melting point) under 400 rpm stirring for 5 min until the solution was completely transparent. The total mass of the solution was controlled to be 100 g while the gel concentration falls in the range of 0.5 to 2.0 wt%. 20 µL ethidium bromide (EtBr) was added into the solution as a fluorescent dye at about 60 °C, which was excited at 210 nm and 285 nm, and emitted orange light with wavelength 605 nm. The solution was poured into a 3D-printed polylactic acid (PLA) mold and allow it cooled for 10-15 minutes to form hydrogel. Then the gel was placed in a transparent horizontal electrophoresis chamber with TAE buffer solution.

The molds and combs used to prepare the agarose gel were designed with Autodesk 2018 and printed by 3D printer (Makerbot Replicator+, USA) using polylactic acid (PLA). The dimension of the chamber is 10×6×1 cm³. Combs with one notch and multiple notches were printed to be used in different purposes.

3.2 Photothermal system

An ultra-high power (100 W) light source (Prizmatix, USA) in visible and infrared range was used to generate temperature gradient in agarose gel. A digital micromirror device (Epson) was used to dynamically control the light patterns and the brightness by program. The surface of the chamber in direct contact with the hydrogel was painted black to absorb incoming light and heat up the gel. Steady temperature gradient was generated by continuously projecting the patterned light on the gel, while transient one by dynamically tuning the heated zone along with the migration of DNA. The surface temperature of hydrogel was recorded by using an infrared
camera (FLIR T430sc) operated at a wavelength of 9-13 μm and image resolution of 320×240 pixels, and analyzed using FLIR tools+ software.

The emissivity of both the black receptor (roughened black paperboard) and the agarose gel are measured first. The emissivity of black receptor is calibrated using a piece of white paper (ε=0.90, wavelength=8-14 μm) as a reference. The black receptor is heated to the same temperature as the white paper and the temperature presented in the infrared camera is adjusted to be the same by changing the emissivity value in the FLIR tool+ software, which is the actual emissivity of the paperboard at this temperature. The dependence of emissivity on temperature is obtained by heating the paperboard to the temperature from 30 to 150 °C. The emissivity of agarose gel is calibrated in similar method by tuning the emissivity value in infrared camera to be the same as its real temperature. The real temperature of agarose gel is directly measured by a thermal couple inserted into the gel near the surface. The dependence of emissivity on temperature is obtained by heating the paperboard to the temperature from 30 to 70 °C. Higher temperature may lead to the melting of agarose gel. Since the detection wavelength of our infrared camera is 8-14 microns, so the measured emissivity is the average emissivity within this range.

3.3 DNA migration on photothermally enhanced gel electrophoresis
Figure 12. Enhanced gel electrophoretic separation of DNAs in dynamically controlled photothermal temperature gradient.

Figure 12 shows the scheme of the temperature gradient electrophoresis system. The gel electrophoresis of dsDNA and ssDNA were tested at a constant voltage of 100 mV for 1-4 hrs. DsDNA were extracted from T lymphoblast cells from human (CCRF-CEM, ATCC). 10 ml of cell suspension at concentration of 0.92 million/ml was used as DNA source. Extraction was carried out using commercial Genomic DNA Mini Kit and following the vender instructions. ssDNA ladders (200 to 2,000 base pair) were used to determine migration rate and ratio of enhancement of temperature gradient electrophoresis. The separation bands were imaged with a fluorescence imaging system (PXi 6, Syngene, UK) under 210 nm ultraviolet excitation.

3.4 Characterizations

3.4.1 UV-vis light transmission

UV-vis light transmission of agarose gel with different concentration and temperature was measured in-situ with a self-built UV-vis spectrometer (Figure 13). All the parts were purchased from Ocean Optics (USA). A UV-vis light source with a wavelength of 200-850 nm was used to generate continuous light passing through the sample. A UV-vis light detector was used to detect the light intensity of the incident after passing through the sample. Optic fibers were used to connect the light source, sample cell and the detector. Two shading boards were used to fix the sample and prevent the disturbance from environmental light. A thermal couple was directly inserted into the sample and the depth was controlled just above the light channel to reduce the measurement error.
Figure 13. In-situ UV-vis light transmission measurement of agarose gel in gelling process.

Boiled agarose solution was placed in the plastic sample cell to measure the light transmission during its gelling process. The thermal couple was tapped to fix its height within the solution. In the induction period, one point is taken every 5 °C; in the fast gelling period, points are taken every 0.5 °C until reaching the room temperature. In the melting process, a high power LED light source with a fixed wavelength at 550 nm was used to heat up the gel. One side of the sample cell was paint to be black as the receptor of the optical energy (Figure 14). Similar measurement procedure was conducted to record the light transmission from room temperature up to around 95 °C.
3.4.2 Mechanical strength

The stress-strain curve of agarose gel at different concentration is measured by ElectroForce Mechanical Test Instrument (TA Instrument, USA). Agarose gel with different concentration from 0.5 to 2.0 wt% were casted and cut into small columns with a dimension of 5×5×20 mm³. The temperature of the agarose gel is controlled by a water bath at desired temperature. After reaching thermal equilibrium, the gel column is quickly taken out for measurement. Since the measurement takes a very short time, the temperature decrease within the gel is ignored. The strain of the test was controlled in the range of 0 to 30% and repeated for three times. The Young’s modulus of the agarose gel can be obtained from the slope of the stress-strain curve. Since the Young’s modulus describes the mechanical strength of the material within its elastic deformation, thus the slope of the stress-strain curve in the range of 0 to 5% was used to determine the Young’s modulus.

3.4.3 Thermal conductivity

Agarose gel with concentration from 0.5 to 2.0 wt% and a dimension of 5×5×20 mm³ are used. An infrared camera (FLIR T430sc) operated at sensitive wavelength of 8-13 µm, image resolution of 320×240 pixels and distance of 0.5 m is used to record the temperature distribution on the gel surface during heating process with an accuracy of ±1 °C. The gel columns are placed vertically on a hot surface maintained at 50 °C. The emissivity of metal surface is calibrated using a piece of white paper (emissivity of 0.90 at 8-13 µm wavelength) as a reference.

A heat transfer model is set up to simulate the temperature profile on the surface of the gel columns. The heat loss around at the surface of the gel by air convection has been considered. A
general one-dimensional heat transfer equation (Eq. 11) is used to describe the temperature profile on the gel surface:

\[
\rho C_p \frac{\partial T}{\partial t} = \frac{\partial}{\partial x} \left[ k(T) \frac{\partial T}{\partial x} \right] - \frac{h(T - T_{air})}{d}
\]  

(11)

where \( \rho, C_p, \) and \( k \) are the density, heat capacity, and thermal conductivity of the agarose gel; \( h \) is the heat convection coefficient of metal sheet in the stagnant air; \( T \) and \( T_{air} \) are the temperatures of the gel and the environment; \( x \) is the distance away from the heat source; \( d \) is the thickness of the gel.

The initial condition and boundary conditions needed to solve this second order non-homogeneous partial differential equation are listed below:

\[
T = T_{air}, @ t \leq 0, x \in [0, L]
\]

(12)

\[
-k \frac{\partial T}{\partial x} = h_{c, eff} (T - T_h), @ x = 0, t > 0
\]

(13)

\[
-k \frac{\partial T}{\partial x} = h(T - T_{air}), @ x = L, t > 0
\]

(14)

where \( h_{c, eff} \) is the thermal contact conductance or the heat transfer coefficient between hot surface and gel column. The conductance can be derived from temperature difference between hot surface and gel at steady state by adjusting the effective heat transfer coefficient in simulation until the temperature of hot surface and metal sheet matches with the experimental result.\textsuperscript{128-129} Finite element analysis is applied to solve the differential equation numerically. The thermal conductivity of the gel at different concentrations are determined by tuning its value in the simulation to match with the experimental measurement.
4.0 PHOTOTHERMAL TEMPERATURE GRADIENT BUILDUP

This chapter discusses the setup of photothermal system controlled by digital micromirror device. The performance of temperature control using the photothermal system will be verified on both black receptor and agarose gel. Black receptor is used as an ideal black body to evaluate the maximum temperature range generated by the light source. Temperature control on agarose gel will be tested to find the optimal condition for gel electrophoresis.

4.1 Calibration of emissivity of black receptor and agarose gel

Before any infrared measurement, the emissivity should be calibrated to obtain the real temperature from the infrared camera. The emissivity of both the black receptor (roughened black paperboard) and the agarose gel are measured first. The emissivity of black receptor is calibrated using a piece of white paper ($\varepsilon=0.90$, wavelength=8-14 μm) as a reference. The black receptor is heated to the same temperature as the white paper and the temperature presented in the infrared camera is adjusted to be the same by changing the emissivity value in the FLIR tool+ software, which is the actual emissivity of the paperboard at this temperature. The dependence of emissivity on temperature is obtained by heating the paperboard to the temperature from 30 to 150 °C. The emissivity of agarose gel is calibrated in similar method by tuning the emissivity value in infrared camera to be the same as its real temperature. The real temperature of agarose gel is directly measured by a thermal couple inserted into the gel near the surface. The dependence of emissivity on temperature is obtained by heating the paperboard to the temperature from 30 to 70 °C. Higher temperature may lead to the melting of agarose gel. Since the detection wavelength of our infrared camera is 8-14 microns, so the measured emissivity is the average emissivity within this range.

Figure 15 shows the temperature dependent emissivity of both black receptor and agarose gel. In Figure 15A, the emissivity of black receptor slowly increases with the temperature from
0.97 to about 0.98. For the convenience of measurement, the average emissivity of 0.975 is used for all the temperature range. Similarly, the emissivity of agarose gel in four different concentrations fluctuate in the range of 0.88 to 0.92 (Figure 15B), so an average of 0.90 is used for agarose gel within the temperature range from 25 to 70 °C.

![Figure 15. Calibration of emissivity on black receptor (A) and agarose gel (B) at different temperature.](image)

**4.2 Temperature control on black receptor**

The temperature range generated by the photothermal system is first calibrated on a black receptor. A roughened black paperboard is used as an absorber since the paperboard has a low reflectance due to its rough surface and has a high absorbance due to the black color. This test illustrates the maximum temperature range this system can achieve. The brightness and the distance from the source are two factors that affect the size and temperature of the hot area. Figure 16 shows the infrared images of temperature patterns on black receptor with different distance from the light source \(d\) and brightness \(b\). At \(d = 12\) cm, the hot area is relatively large while the temperature is relatively low. When the brightness is 100%, a symmetric patterns is shown (Figure 16A). When the brightness varies from 100 to 50% and from 50 to 5%, the hot area becomes asymmetric and the center temperature decreases from 70.3 to 46.3 °C (Figure 16B, 16C). When
the distance decreases to 6 cm, the hot area becomes smaller but the temperature increases, since the total energy put into the gel keeps the same for certain brightness. The maximum temperature could reach nearly 130 °C for 100% brightness and a large temperature gradient can be achieved (Figure 16D). When the brightness varies from 100 to 50% and from 50 to 5%, similar asymmetrical patterns can be observed. But compared to \(d=12\) cm, the center temperature is much higher (Figure 16E and 16F).

![Infrared images of temperature patterns on black receptor with different distance from the light source (d) and brightness (b). (A) \(d=12\) cm, \(b=100\%\); (B) \(d=12\) cm, \(b=100-50\%\); (C) \(d=12\) cm, \(b=50-5\%\); (D) \(d=6\) cm, \(b=100\%\); (E) \(d=6\) cm, \(b=100-50\%\); (F) \(d=6\) cm, \(b=50-5\%\).](image)

**Figure 16.** Infrared images of temperature patterns on black receptor with different distance from the light source (d) and brightness (b). (A) \(d=12\) cm, \(b=100\%\); (B) \(d=12\) cm, \(b=100-50\%\); (C) \(d=12\) cm, \(b=50-5\%\); (D) \(d=6\) cm, \(b=100\%\); (E) \(d=6\) cm, \(b=100-50\%\); (F) \(d=6\) cm, \(b=50-5\%\).

The surface temperature distribution on agarose gel is extracted from the infrared images in Figure 16 and shown in Figure 17. The temperature at center decreases from 128, 95 to 57 °C at a distance of 6 cm and from 85, 70 to 46 °C at a distance of 12 cm when the brightness of the light source varies from 100% to 5%. While the temperature filed length with a distance of 12 cm is almost twice of that with a distance of 6 cm. The colored bars in the legend qualitatively indicate the temperature range and field area in each condition. This method can be applied to the receptors with varied dimensions by tuning the distance between the light source and the receptor.
Figure 17. Temperature distribution on agarose gel with different distance from the light source and brightness.

**4.3 Temperature control on agarose gel**

The photothermal system was used to control temperature on an agarose gel with a size of 6×10×0.5 cm³. Figure 18A-18F shows the infrared images of surface temperature distribution on agarose gel with different programmed light patterns at 6 cm from the light source, where the length of heated zone keeps the same (5-6 cm) for each case. The single brightness configuration can be used to generate uniform temperature gradient with different temperature range controlled by the value of brightness. At the lowest brightness (5%), a symmetric temperature gradient is shown on the gel with a temperature of 24.9 °C (Figure 18A); at the highest brightness (100%), a high contrast temperature gradient is shown with the highest temperature of 60.8 °C (Figure 18B). A continuously varied brightness configuration can be used to create a temperature field with larger gradient ranging from room temperature to nearly 60 °C (Figure 18C). Based on the flexible tuning capability of DMD system, separated control of temperature gradient on multiple paralleled channels (Figure 18D-E) or at certain regions (Figure 18F) have also been validated. Dynamic
temperature gradient has been achieved by programming a movable light band with tunable velocity through the control of DMD system. Based on the output voltage applied in gel electrophoresis, the velocity of the movable light band can be matched with different migration rate of DNA.

Figure 18. Infrared images of surface temperature gradient on agarose gel with different light brightness and patterns. (A) Brightness=5%; (B) brightness=100%; (C) brightness=5~100%; (D)-(F) multiple temperature gradients generated by varying brightness from 5 to 100% controlled by DMD.

Surface temperature distributions of agarose gel under different brightness are extracted from the infrared images (Figure 18A-C) and shown in Figure 19A. With a fixed brightness (5% or 100%), the temperature distribution uniformly stays in a small range. When the brightness varies from 5 to 100%, the surface temperature can be tuned from room temperature to above 60 °C within the light window with a dimension of 6 cm×4 cm. To better predict the thermal effect at different brightness, the luminance of the light is measured to relate to the surface temperature
shown in Figure 19B. Both the brightness and the luminance increase quasi-linearly with the surface temperature, which helps in quick prediction of the surface temperature without reaching thermal equilibrium, especially when distance from the light source is changed.

![Graphs showing temperature, brightness, and luminance relationships](image)

**Figure 19.** Areas and ranges of temperature gradient on agarose hydrogel (A); dependence of gel temperature on light brightness and luminance (B); temperature increase of ink heated with different light brightness (C); dependence of output power and power density of the light source on the brightness (D).

The power density of the light source with different brightness is calibrated with blank ink solution, which is considered to have a light absorbance of about 0.95 in the visible light range.\textsuperscript{130} The ink solution is placed in a beaker surrounded with thermal insulator to prevent the heat loss from the beaker walls. The temperature increase of the ink water under the high-power light source is recorded in Figure 19C. Obviously, the temperature increase presents a linear relation with the heating time and the slope is proportional to the light brightness. With the temperature increase
rate under different brightness, the output power of the light source is calibrated in Figure 19D.
The power density per unit area is calculated based on the output power. The power density can reach about 500 W/m$^2$ at 100% brightness, which is about half of the power density from the sun. This number can be used to estimate the energy consumption of the enhanced separation process.

4.4 Heat transfer along the direction of thickness of the gel

![Figure 20. Temperature gradient spread in the direction of the thickness. Infrared image of agarose gel faced to (A) and back on to (B) the light energy.](image)

The heat conduction through the thickness of the gel may affect the accuracy of the temperature control. If a heat flow pass through a wall, due to the thermal resistance, there will be a temperature difference between two sides of the wall. In the photothermal system, the heat comes from the bottom to the gel while the DNA migrates within the gel, heat will conducted through the thickness of the gel to “dilute” the temperature gradient. In heat transfer, a dimensionless number, Biot number is always used to describe the effect of heat transfer in the wall. When Biot number is smaller than 0.1, the wall can be regarded as thermally thin and the temperature gradient within the wall can be ignored. When larger than 0.1, thermal resistance of the wall has to be considered. The surface temperature of both sides of the gel are recorded by the infrared camera and analyzed in Figure 20. The result indicates that both the temperature distribution and the area of heated zone are almost the same on the two sides, except on the back, the temperature gradient is not as clear.
as on the side directly facing the light energy. Since the thickness of the gel is only 0.5 cm and the thermal conductivity of agarose hydrogel is 0.51-0.65 W/m·K, the Biot number of the gel is 0.05-0.10, which means the gel film can be regarded as “thermally thin”, namely the temperature gradient within the gel can be ignored without suffering an error larger than 5%.

4.5 Summary

In this chapter, DMD controlled photothermal system was built up and calibrated in both black receptor and agarose gel. Temperature gradient between 20-60 °C was established in gel with dynamically controlled patterns. The relation between light brightness and temperature, luminance, and power density was measured for quantitative analysis. While the effect of gel thickness can be ignored in heat transfer.
5.0 TEMPERATURE EFFECT ON GEL PROPERTIES

Having proved the temperature control with DMD assisted photothermal system, the influence of temperature on different properties of agarose gel is of interest, including phase behavior, pore size, mechanical strength, and thermal conductivity. These properties will affect the DNA migration in the gel electrophoresis. Knowing the dependence of those properties on temperature will help to accurately control the DNA separation with photothermal enhanced gel electrophoresis.

5.1 Temperature effect on phase transition of agarose gel

The agarose gel is a thermally reversible system, which forms the gel at low temperature and becomes back to solution at high temperature. So, the temperature influence during the phase transition of agarose gel is studied first. The phase transition of the gel can be indicated by the UV-vis light transmission since when it forms gel, it becomes partially opaque.

Figure 21 shows the UV-vis light transmission of agarose gel with a concentration of 0.5, 1.0, 1.5, and 2.0 wt% at different temperature in the gelling process. In the gelling process, the light transmission keeps high (>90%) in the solution phase after melted for all the four different concentrations. When temperature falls down to about 40 °C, the light transmission starts to decrease. The abrupt change of light transmission happens at 31.0, 33.0, 34.6, and 35.7 °C for agarose gel with a concentration of 0.5, 1.0, 1.5, and 2.0 wt%, respectively. After reaching the abrupt change point, the light transmission quickly decreases in the following 10 °C. Then the decrease slows down and stops at the room temperature. Beside the abrupt change point, the light transmission after the formation of the gel is also different in four concentrations. If the maximum transmission at around 490 nm is used to indicate the phase transition process, the light transmission is 65.4%, 44.8%, 34.6%, and 33.1% for agarose gel with a concentration of 0.5, 1.0,
1.5, and 2.0 wt%, respectively. Agarose gel is actually a two-phase system: polymer chains and water trapped inside. When the concentration of agarose is high, when forms the gel, more polymer chains are existed in the gel, which absorbed more light. The decrease of light transmission with the concentration is obvious from 0.5 to 1.5 wt%, but becomes insignificant from 1.5 to 2.0 wt%. It is inferred that the number of polymer chains reaches its saturation for light absorbance at 1.5 wt% concentration, when the concentration further increases to 2.0 wt%, its contribution to light absorbance is reduced.

![Figure 21](image1.png)

**Figure 21.** UV-vis light transmission of agarose gel with a concentration of 0.5 wt% (A), 1.0 wt% (B), 1.5 wt% (C), and 2.0 wt% (D) at different temperature in the gelling process.

Similar measurement is conducted during the melting process of the gel. Figure 22 shows the UV-vis light transmission of agarose gel with a concentration of 0.5, 1.0, 1.5, and 2.0 wt% at different temperature in the melting process. For each concentration, the transmission continuously
decreases when the gel is heated from room temperature to about 65-75 °C. This is due to the swelling phenomenon during the heating process before melting, where the polymer chains are partially melted. After reaching the melting point, the light transmission suddenly increases from low level to high level for another 10 °C. After completely melted, the light transmission returns back to the initial state before gelling. Since the transmission is low in the gel phase at high concentration, so the difference before and after melting is also significant. Different melting points have been observed at different gel concentration. The melting process starts at 62.3, 68.2, 70.4, and 75.8 °C and ends at 79.4, 82.5, 85.9, and 87.6 °C for agarose gel with a concentration of 0.5, 1.0, 1.5, and 2.0 wt%, respectively. This is the reverse effect of gel concentration on gelling point, since higher concentration forms denser polymer chains, which takes more heat to break them up.
Figure 22. UV-vis light transmission of agarose gel with a concentration of 0.5 wt% (A), 1.0 wt% (B), 1.5 wt% (C), and 2.0 wt% (D) at different temperature in the melting process.

To better illustrate this process, the maximum transmission at about 490 nm is extracted at different stages and plotted here. For different gel concentrations, similar hysteresis loop can be obtained in Figure 23. Compared the loops in different concentration, it is found that at low concentration, the area of the loop is much smaller, which means the gel looks more transparent at low concentration since the transmission is higher. This is because agarose gel is actually a two-phase system, polymer chains and water trapped inside. When the concentration is low, less polymer chains absorb less light, so it looks clearer. Second, the gelling point and melting point decrease with the concentration. This can be explained by the nucleation and growth mechanism in crystal theory (Figure 24). In the gelling process, the agarose experiences induction period,
gelling period and aging period. Before gelling, agarose is dissolved in the water. When temperature decreases, small particles start to form and grow in the solution, which is called the induction period. When the size of the particle reaches the critical nucleation size, it quickly precipitates from the water phase and cross link with other chains. At the late gelling stage, gel is formed with sparse polymer chains. In the aging stage, the polymer chains become denser with less detects. The critical nucleation size is affected by the concentration and the temperature. At high concentration, it is easier to reach the critical size at a higher temperature since more particles can be formed and they grow faster.

![Diagram](image)

**Figure 24. Nucleation and growth mechanism in gelling and melting process of agarose gel.**

In the melting process, the gel experiences swelling process, melting process and finally becomes transparent solution. First, the gel starts to swell and the polymer chains are partially broke up when temperature increases. When temperature becomes higher, the distance between the chains are large enough and the attraction between chains is not strong enough to maintain the
gel structure. The cross-linked structure will completely break up into short polymer chains. Then the chains will gradually dissolve in the aqueous solution.

5.2 Temperature effect on gel pore size

The transmission of the gel is related to its correlation length or the so-called pore size. The temperature dependent pore size can be calculated from the water turbidity. Figure 25 shows the process of the determination of pore size of agarose gel from light absorbance.

Figure 25. Determination of agarose gel pore size from water turbidity. Absorbance and water turbidity of agarose gel in 700-800 nm wavelength under different temperature (A); determination of wavelength exponent from wavelength dependent water turbidity (B); determination of pore size under different temperature with wavelength exponent (C).

The absorbance of agarose gel between 700 and 800 nm can be obtained from light transmission spectrum for different gel concentration under different temperature. The absorbance is used to calculate the water turbidity at each wavelength (Figure 25A) based on:

\[ \tau(\lambda) = \frac{2.3A(\lambda)}{L} \]  

(15)

where \( \tau \), \( A \), \( \lambda \) and \( L \) are turbidity, absorbance, wavelength and optical path length (\( L = 1 \ cm \)), respectively. Both absorbance and turbidity decrease with wavelength at the same temperature, and increase with temperature for each wavelength. The wavelength exponent (WLE) is obtained
from the linear regression of a double logarithmic plot of turbidity vs wavelength shown in Figure 24B. The turbidity of a solution of monodisperse particles at a given wavelength ($\lambda$) is:

$$\tau(\lambda) = H(\lambda) \cdot Q(\lambda) \cdot S(\lambda) \cdot M \cdot c$$  \hspace{1cm} (16)

where $M$ and $c$ are molecular weight and particle concentration; $H(\lambda)$, $Q(\lambda)$ and $S(\lambda)$ are called optical constant function, interparticle correction function and intraparticle dissipation factor, respectively. Eq. (16) can be simplified to:

$$\frac{d \log \tau(\lambda)}{d \log (\lambda)} = - \left( 4 + \alpha_1 + \alpha_2 + \frac{d \log Q(\lambda)}{d \log (\lambda)} \right)$$  \hspace{1cm} (17)

$$\alpha_1 = 2 \frac{d \log (n_0)}{d \log (\lambda)}, \alpha_2 = 2 \frac{d \log (dn/dc)}{d \log (\lambda)}$$  \hspace{1cm} (18)

where $\alpha_1$ and $\alpha_2$ are 0.0248 and 0.0922 for wavelength between 700-800 nm, respectively. Figure 25C indicates the measured relation between WLE and pore size, where three regions can be observed: the pore size is independent of WLE with a constant value of -4.117 and -2.117 for pore size smaller than 10 nm or larger than 5 μm; the wavelength exponent of hydrogel increases rapidly with the pore size in intermediate range. This correlation is based on the assumptions that fibers constructing the gels are flexible when they are smaller than the pore size, and the scattering contribution of their local structure can be ignored. WLE values at different temperature calculated from Figure 25B are used to find the according pore size of the gel. As temperature increases from 25, 40, 50 to 60 °C, the pore size of the gel increases from 231, 285, 330 to 369 nm. The increased pore size reduces diffusion resistance and enhances migration rate of DNAs.
Figure 26. Pore size variation in gelling (A) and melting (B) process of agarose gel with different concentration.

Based on the calculation procedure, pore size variation in gelling and melting process of agarose gel with different concentration is obtained in Figure 26. During the induction of the gelling process, no pores can be detected, since the small gel particles (nuclei) cannot form any cross-link structure at the moment. In this stage, the pore size (or the correlation length) is defined as the distance between gel particles. While when the gel formation starts, the pore size quickly increases. When it reaches the aging period, this decrease slows down and stop eventually. The final pore size decreases with the increase of concentration, since more polymer chains are existed at high concentration and denser cross-link structure with small pores is formed.

In the application of DNA separation, the melting process attracts more interest. In Figure 26B, the pore size increases linearly with the temperature before reaching the breakup point. Since the resistance for DNA in electrophoresis comes from the pores of the gel, which can distinguish DNA with different length. We can easily control the resistance of DNA migration just by heating up certain area we are interested in. Furthermore, Figure 26B also informs that the temperature range has to be controlled within the manageable range: at 0.5 wt% concentration, the temperature
must be controlled below 60 °C, while at 2 wt%, the temperature range can be extended to about 75 °C.

5.3 Temperature effect on mechanical and thermal properties

Temperature dependent of other properties are also measured. This figure shows the stress-strain curves of agarose gel at different concentrations and temperatures. Young’s modulus can be derived from stress-strain curve by getting its slope in the small strain range when it remains in the elastic deformation. When concentration decreases from 2 to 0.5%, the mechanical strength decreases rapidly, while temperature has a relative mild influence on it. When temperature increase, the mechanical strength slightly decreases due to the partially breakup of the polymer chain structures. This small change would not generate disruptive structure change to impair the separation of DNA.

![Figure 27](image)

**Figure 27.** Temperature dependent mechanical property of agarose gel at different concentration. Stress and strain curve of agarose gel (A); Young’s modulus as a function of gel concentration and temperature (B).

Thermal conductivity of agarose gel is also measured at different concentrations with the infrared imaging system based on the heat transfer theory. The measured thermal conductivity is
the average in the temperature range of 20-60 °C. The result shows that the thermal conductivity decreases slight with the concentration increase. The relatively low thermal conductivity of agarose gel helps to maintain the thermal gradient within the gel and it takes longer to transfer the heat into the surrounding solution. This prolongs the operation of photothermal enhanced electrophoresis.

Figure 28. Temperature dependent thermal conductivity of agarose gel at different concentration. Infrared images of top view (A) and front view (B) of agarose with concentration of 0.5 (1), 1.0 (2), 1.5 (3), and 2.0 (4); thermal conductivity as a function of agarose concentration (C).

5.4 Summary

In this chapter, phase behavior of agarose gel was observed by in-situ measuring the light transmission and the hysteresis loop is obtained. Nucleation and growth mechanism was used to describe the gelling and melting process. Pore size of the agarose gel was then calculated based on the transmission measurement and found it increases with the temperature and decreases with the concentration. The temperature effect on mechanical strength and thermal conductivity will facilitate the operation of photothermal enhanced electrophoresis.
6.0 PHOTOTHERMAL ENHANCED GEL ELECTROPHORESIS FOR DNA SEPARATION

6.1 DNA migrations in static and dynamic light control

The effect of temperature on dsDNA migration has been explained as that high temperature would partially denature the double helix structure to increase the electric charge of DNAs due to more free nucleobases are created, leading to a higher migration rate in electric field. However, this hypothesis can hardly explain the temperature effect on ssDNA ladder or small molecules, such as Bromophenol blue and xylene cyanol, which are used as color markers to trace DNA migration. High temperature may not create more electric charges for ssDNA and small molecules. Since the bands of different molecules (dsDNA, ssDNA and dye molecules) under the same temperature gradient present similar curvature, it is inferred that certain physical property of agarose gel may be affected. Pores in the gel is the source of resistance for DNAs with different length, which determines their migration rates. The pore size of the gel can be easily affected by temperature due to thermal expansion, which will further change the migration rate of DNAs.

DsDNA is directly separated from cells with a length of about 20,000 bp and used to test the effect of temperature gradient on migration rate. Figure 29 shows the migration of dsDNA in agarose gel electrophoresis with and without temperature gradient. Temperature remains at 20 °C on the left and a steady temperature gradient from 20 to 60 °C is applied on the right. After migration for 1 h, no obvious difference is observed on both sides with a nearly straight band; a shift starts to appear on the right after 2 h and a horizontal “S” shape band is observed after migration for 3 and 4 hours. The curvature in the DNA band indicates that the migration has been accelerated when the gel is heated.
To quantify the migration of DNA under the temperature gradient, commercial ssDNA ladder with 10 bands from 200 to 2,000 bp is applied. Figure 30A shows the optical image of ssDNA ladder after migration for 1.5 h with ssDNA length labeled for each band. Curved DNA bands are observed for all DNA fragments and the curvature increases inverse proportionally to DNA length. The larger migration distance and curvature presented in short DNA bands indicates that they are subjected to smaller resistance from the pores of hydrogel and more sensitive to the temperature. Figure 30B shows the migration rate of different DNA fragments with and without heating. The fastest migration point is chosen as the representative for the heated area and the slowest as the control area. The migration rate presents a good linear correlation with DNA length for both heated and control area. The absolute value of the slope for heated area is larger than that of control area, indicating that the separation capability of electrophoresis is remarkably enhanced with the assistance of photothermal heating.
Figure 30. Optical image of hydrogel electrophoresis of ssDNA ladder under static temperature gradient after migration for 1.5 h (A); dependence of migration speed on temperature and DNA length (B).

In order to further enhance the separation capability and determine the enhancement ratio, a dynamic temperature gradient is applied, where the light source is moved with the DNA migration. The dynamic temperature gradient provides a relatively constant environment for DNAs, where they experience identical temperature gradient during migration. Figure 31A shows an optical image of hydrogel electrophoresis after migration for 1.5 h under the dynamic temperature field. Along the red line, DNA always migrates under the temperature of 60 °C and same for yellow and green lines. This indicates that the migration rates of all fragments are faster than those under a static temperature gradient. From the data shown in Figure 30B, the migration rate reaches 0.90 mm/min for DNA fragment with 200 bp in length, which is 0.75 mm/min under steady temperature gradient (Figure 31B). Both the migration rate and the slope increase with the temperature, indicating a better enhancement of migration with transient temperature field. Enhancement ratio is defined to quantify the effect of photothermal heating on separation capability of gel electrophoresis as follow:
\[ \text{Enhancement ratio} = \frac{v_{\text{max}} - v_{\text{min}}}{v_{\text{min}}} \]  

The result is shown in Figure 31C, where when the length of the fragment is less than 400 bp, static temperature field gives higher enhancement ratio; while longer than 400 bp, dynamic one performs better. The dynamic temperature gradient electrophoreses is especially efficient for the separation of longer DNA fragments: when the length is 2,000 bp, the enhancement ratio is 25% higher. If the linear relation is extrapolated to 20,000 bp, the enhancement ratio will reach 205.9% in the transient temperature gradient electrophoresis, which significantly increases its separation capability. In the DNA separation, the gel temperature will be controlled uniformly with a larger light source to acquire a straight band for high-quality separation.

Figure 31. Optical image of hydrogel electrophoresis of ssDNA ladder under dynamic temperature gradient after migration for 1.5 h (A); dependence of migration rate on DNA length at temperature of 30, 45, and 60 °C (B); comparison of enhancement ratio in static and dynamic temperature gradient (C).
6.2 Effect of electrophoresis output voltage on DNA migration

Figure 32. DNA migration in 1 wt% agarose gel electrophoresis under the voltage of 50 (A), 80 (B), 100 (C), 120 (D), and 150 mV (E) for 1 h.

Having determined to use the dynamic light control, DNA migration under different voltages is studied first. Figure 32 shows the DNA migration in 1 wt% agarose gel electrophoresis under the voltage of 50, 80, 100, 120, and 150 mV for 1 hour. Obviously, the higher voltage is applied, the faster the DNA migrates, since the electric field is the driving force in electrophoresis. While in each picture, the curve bands can still be observed and it seems that the temperature has a better enhancement for high voltage. From the DNA migration images, the mobility of DNA can be calculated by dividing the distance travelled in the gel to the time of migration.

Figure 33 shows the DNA length dependent electrophoretic mobility in 1 wt% agarose gel electrophoresis under different output voltages. At each voltage, the DNA mobility decreases linearly with the length increases from 200 to 2000 bp. For the same length of DNA, the mobility increases with the temperature, since high temperature will generate large pore size within the gel, where the resistance for DNA migration will be reduced. Compared the mobility at different voltages, the general mobility of DNA with different lengths increase with the increasing voltage. The mobility of 200 bp DNA fragment at 60 °C reaches 1.35×10^{-4}, 1.42×10^{-4}, 1.47×10^{-4}, 1.51×10^{-4}.}

54
4, and 1.53×10⁻⁴ cm²·V⁻¹·s⁻¹ at a voltage of 50, 80, 100, 120, and 150 mV. However, it has a trivial influence on the longer DNA (from 1600 to 2000 bp) when the voltage is as low as 50 mV. Even when the voltage increases from 80 mV to 150 mV, the influence seems to be not as significant for longer DNA as the shorter ones.

Figure 33. DNA length dependent electrophoretic mobility in 1 wt% agarose gel electrophoresis under the voltage of 50 (A), 80 (B), 100 (C), 120 (D), and 150 mV (E).

By measuring the mobility for different voltages, the dependence of mobility as a function of voltage at certain temperature is obtained in Figure 33. At each temperature, the mobility increases linearly with the voltage for different lengths. When temperature increases from 30 to 60 °C, the mobility of DNA in all lengths increases. In this group of experiment, both voltage and temperature affect the migration mobility of DNA. In order to obtain the influence by temperature itself, each straight line can be extrapolated to voltage at 0 mV, where the intrinsic mobility of DNA at different temperature is obtained in Figure 34. This value shows the influence of temperature only on the migration rate of DNA with different lengths.
Figure 34. Output voltage dependent electrophoretic mobility of DNA with length from 200 to 2000 bp under 30 (A), 45 (B) and 60 °C (C).

From Figure 35, it is clearly seen that the intrinsic mobility increases with temperature but decreases with the DNA length. It seems that when DNA is short, the increase is more evident. However, if the relative increase ratio is compared, longer DNA has a higher ratio since the mobility for longer DNA is small, a tiny increase in mobility will give out a larger increase ratio. The relative increase ratio or called the enhancement ratio will be used to evaluate the temperature effect on the mobility enhancement for different DNA length later.

Figure 35. Temperature dependent intrinsic electrophoretic mobility of DNA fragments with different lengths.
6.3 Effect of gel concentration on DNA migration

Figure 36. DNA migration in agarose gel electrophoresis with concentration of 0.5 (A), 1.0 (B), 1.5 (C), and 2.0 wt% (D) under the voltage 100 mV for 1 h.

Then the effect of concentration on DNA migration with temperature gradient is studied. Figure 36 shows the DNA migration in agarose gel electrophoresis with concentration of 0.5, 1.0, 1.5, and 2.0 wt% under the voltage 100 mV for 1 h. As have discussed before, the pore size of the agarose gel decreases with the concentration increase, so the migration rate will be lower at higher concentration. Temperature gradient still plays a key role in the DNA migration. Similar analysis on migration mobility is done for different gel concentrations.

Figure 37 shows the concentration dependent electrophoretic mobility of DNA with length from 200 to 2000 bp under 30, 45 and 60 °C. First, the migration mobility as a function of agarose concentration at different temperature shows that the mobility always follows a linear relation when the concentration increases at each temperature. When temperature increases from 30 to 60 °C, the mobility at each concentration increases to different extent. For example, the mobility of DNA with a length of 200 bp increases from $1.39 \times 10^{-4}$, $1.54 \times 10^{-4}$, and $1.68 \times 10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$ at
temperature of 30, 45, and 60 °C for a concentration of 0.5 wt%. Similar trend has been observed in other three concentrations.

Figure 37. Concentration dependent electrophoretic mobility of DNA with length from 200 to 2000 bp under 30 (A), 45 (B) and 60 °C (C).

Figure 38. Temperature dependent electrophoretic mobility of DNA with length from 200 to 2000 bp with concentration of 0.5 (A), 1.0 (B), 1.5 (C), and 2.0 wt% (D).

Figure 38 shows the temperature dependent electrophoretic mobility of DNA with length from 200 to 2000 bp with concentration of 0.5, 1.0, 1.5, and 2.0 wt%. Except the linearly increase of
DNA mobility with temperature for all four concentrations, the influence of temperature on each lengths draws more attention. In each concentration, the slope of the straight line increases with the DNA length. As the concentration increases, the slope change as the increase of DNA length becomes more significant, which indicates that the enhancement of temperature may have a better performance on higher concentration, where the pore size of the gel is smaller. This is easy to be understand that when the pore size is small, the resistance for DNA migration becomes strong. The swelling of gel at low concentration will give a larger increase to the pore size relatively and will relief the resistance to a great extent.

Figure 39. Enhancement ratio of photothermal enhanced gel electrophoresis under temperature of 30, 45 and 60 °C.

The purpose of applying thermal gradient is to increases the separation capability of DNA band from the adjacent ones, so the distance between adjacent bands at different temperature is of interested. To quantify the temperature effect, we use the enhancement ratio as the relative increase in mobility at higher temperature compared to the room temperature. From the temperature dependent mobility, the enhancement ratio at different temperature can be calculated shown in Figure 39. From the figure, the higher temperature has a higher enhancement ratio. For different
length of DNA, longer ones has a better enhancement under the temperature gradient. The enhancement ratio could reach about 70% for 2000 bp DNA. The performance could be better for longer ones, such as 20 kbp DNA fragment. This results is very useful since the limitation of gel electrophoresis is the low separation capability for long DNA. With the help of temperature gradient, higher separation rate can be achieved for long DNA band.

6.4 Photothermally enhanced electrophoresis for DNA separation

![Figure 40](image.png)

*Figure 40. DMD controlled photothermal electrophoresis for whole range (A) and selected range (B) enhancement in DNA separation; sketch of pore size tunability of DMD controlled photothermal system in gel electrophoresis(C).*

In the real application, curved bands are not easily separated. By creating uniform temperature gradient in two separate zones, straight DNA band can be obtained for further separation. In Figure 40A, a group of straight DNA bands is obtained with larger distance in between with a high temperature zone created with 100% brightness on the left side. This enhancement is applied for the whole range of DNA band since a uniform temperature field is created. If certain range of DNA needs to be enhanced, a temperature gradient can be applied just under this region to achieve
selected enhancement (Figure 40B). All the separation enhancement is based on the tuning of pore size with the temperature gradient as illustrated in Figure 40C. The selected enhancement indicates the flexible control on DNA separation and no other techniques can be realized so far. Energy consumption is another concern in the real application. Based on the energy consumption correlation and enhancement ratio in Figure 19D and Figure 39, a power density of 495 W/m$^3$ is required to achieve a 70% enhancement for 2,000 bp fragments.

### 6.5 DNA enrichment by inversely application of temperature gradient

Photothermally controlled temperature gradient can be applied for the enhancement of DNA separation when the fragments move from lower temperature to higher temperature. On the other hand, if the temperature gradient is reversed, the DNA will be enriched when migrated from higher temperature to lower temperature with a large gradient, where the pore size of the gel is abruptly decreased and the resistance increased. Figure 41 shows the DNA enrichment along y axis from 0 to 60 min. At t=0 min, a small cube of agarose gel with uniformly distributed DNA is heated with DMD controlled light source to about 60 °C (Figure 41A). DNA with the same length is used in this case to keep the initial migration rate to be the same. Other than the area containing DNA, no heat is applied. So a great temperature will be generated at the bottom boundary of the DNA concentrated area. When DNA migrates out of its original area, the large resistance at low temperature area will slow down its mobility while DNA in the heated area still keeps at high mobility. In this way, DNA will be enriched at the moving end as time goes on. From Figure 41B to 41E, it is clearly seen that the width of DNA (along x axis) area keeps the same while the height (along y axis) is gradually decreased. When all the DNA molecules move out of the high temperature area, the difference between two ends will be disappeared and the enrichment will be stopped (Figure 41F). At 60 min, the area of DNA decreases to about 1/3 of its initial area.
Figure 41. DNA enrichment along y axis using reverse temperature gradient for 0 (A), 10 (B), 20 (C), 30 (D), 45 (E), and 60 min (F).

The DNA can be further enriched along the other direction by changing the direction of the electric field. The enrichment along x axis is shown in Figure 42. Similarly to the process along y axis, the dimension of the DNA area that is perpendicular to the direction of the electric field will keep the same while it will be shrunk along the direction that is parallel to the electric field. From the intensity of the excited fluorescence in Figure 42B to 42D, the enrichment process can be clearly verified.

Figure 42. DNA enrichment along x axis using reverse temperature gradient for 0 (A), 30 (B), 45 (C), and 60 min (D).
The enrichment ratio of this method can be defined as the final DNA concentration over initial DNA concentration, which is inversely proportional to the fluorescent area by assuming the molecules are evenly distributed and no DNA loss happens. Figure 43 indicates the area change of DNA fragment and the enrichment ratio after migrating in the electrophoresis for 60 min. The initial area of DNA is 1.0 cm² and when the time increases to 1 hour, the area decreases to less than 0.4 cm², while the enrichment ratio reaches as high as 2.7, which means that the concentration of DNA increases to 2.7 times of its initial concentration. After the direction of electric field is vertically changed, the enrichment ratio would reach 5.0 for another 1 hour electrophoresis. The enrichment ratio can be further increased if larger temperature gradient is applied.

![Figure 43. Enrichment factor of DNA fragment along x and y axis for 120 min.](image)

**6.6 Summary**

In this chapter, both double-strand DNA and single-strand DNA marker have been used to verify the photothermal enhanced electrophoresis. Compared to static light control, dynamic light control creates a more uniform temperature gradient along the migration of DNA and the separation capability is higher. Effect of electrophoretic voltage and gel concentration on DNA migration mobility has been discussed and found high voltage and low concentration lead to a
higher migration mobility. With the help of temperature gradient, longer DNA has a better enhancement ratio under certain voltage and concentration. The enhancement effect is especially significant for higher gel concentration. Inverse temperature gradient is also applied for DNA enrichment in two dimensions. The enrichment ratio is affected by the temperature gradient and the enrichment time in the electrophoresis.
7.0 CONCLUSIONS

Agarose gel electrophoresis is one of the most widely used genetic fingerprinting technique in DNA separation, which indicates the genetic diversity of a biological community. Temperature, as one of the control parameters, affects the structural and mechanical properties of gel such as pore size, gelation rate and elastic modulus, among which the pore size has a significant impact on diffusion. This project proposed a photothermal method, combining remote infrared heating, digital micromirror control and infrared imaging, to control the pore size of agarose, hence enhance gel electrophoretic separation of DNAs.

DMD controlled photothermal system was first built up and calibrated in both black receptor and agarose gel. Temperature gradient between 20-60 °C was established in gel with dynamically controlled patterns. The relation between light brightness and temperature, luminance, and power density was measured for quantitative analysis. While the effect of gel thickness can be ignored in heat transfer.

At last, both double-strand DNA and single-strand DNA marker have been used to verify the photothermal enhanced electrophoresis. Compared to static light control, dynamic light control creates a more uniform temperature gradient along the migration of DNA and the separation capability is higher. Effect of electrophoretic voltage and gel concentration on DNA migration mobility has been discussed and found high voltage and low concentration lead to a higher migration mobility. With the help of temperature gradient, longer DNA has a better enhancement ratio under certain voltage and concentration. The enhancement effect is especially significant for higher gel concentration. Inverse temperature gradient is also applied for DNA enrichment in two dimensions. The enrichment ratio is affected by the temperature gradient and the enrichment time in the electrophoresis.
Phase behavior of agarose gel was then observed by in-situ measuring the light transmission and the hysteresis loop is obtained. Nucleation and growth mechanism was used to describe the gelling and melting process. Pore size of the agarose gel was then calculated based on the transmission measurement and found it increases with the temperature and decreases with the concentration. The temperature effect on mechanical strength and thermal conductivity will facilitate the operation of photothermal enhanced electrophoresis. Given the easiness of light modulation, the photothermal temperature gradient can be a powerful way to enhance separation capability of a given gel in electrophoresis.
8.0 REFERENCES


76


