Spectrometric Analysis Using Liquid Metal Mirrors in a Microfluidic Device

Korea Advanced Institute of Science and Technology

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Spectrometric Analysis Using Liquid Metal Mirrors in a Microfluidic Device
Spectrometric Analysis Using Liquid Metal Mirrors in a Microfluidic Device

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Approved by

____________________________
Professor Je-Kyun Park
(Major Advisor)
미세유체소자 내 액체금속거울을
이용한 분광학적 분석

최 재 규

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2009년 12월 22일

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Abstract

This paper presents a new absorbance measurement device using multiple internal reflections and a new novel spectrometer for both of them using fluidic mirrors filled with liquid metal. Liquid metal mirrors reflect and gather light from LED with high reflectivity and small sample volume. Optical path length has been extended with various incident angles of 20°, 30°, 40° and 50°. We demonstrated the enhancements of the sensitivity and limit of detection (LOD) compared to the air mirrors and linear channel. As incident angle lowers, optical path length extension becomes more effective without trade-offs of sensitivity and LOD. This device with incident angle of 50° has a 84 nM of LOD and 3.62 x 10⁻⁴ of sensitivity for absorbance measurement of fluorescein diluted in deionized water. The spectrometer is suitable for direct spectral analysis in the sample channel plane using fluidic slit and mirror filled with liquid metal. Solutions of phenol red and trypan blue which are used widely for biomolecular detection are measured for spectrometric analysis. This platform can be simply adapted to the applications using the fiber optics integration. The measurements of phenol red and trypan blue result 212 nM, 154 nM of LOD and 1.09x10⁻³, 8.10x10⁻⁴ of sensitivity respectively.
Table of contents

Abstract .......................................................................................................................... i
Table of contents .......................................................................................................... ii
Nomenclature ................................................................................................................ iii
List of Tables .................................................................................................................. vi
List of Figures ................................................................................................................ vii

1. Introduction .................................................................................................................. 1
   1.1 Optical measurements in lab chips ............................................................... 1
   1.2 Research objectives ....................................................................................... 11

2. Materials and Methods ............................................................................................. 12
   2.1 Chip design ........................................................................................................ 12
   2.2 Fabrication process .......................................................................................... 18
   2.3 Experimental setup .......................................................................................... 22
   2.4 Evaluation criteria ............................................................................................ 26

3. Results and Discussions ........................................................................................... 28
   3.1 Optical path extension ..................................................................................... 28
   3.2 Spectrometer ...................................................................................................... 28

4. Conclusions ................................................................................................................ 39
   4.1 Summary ............................................................................................................ 39
   4.2 Further works .................................................................................................... 40

Summary in Korean ......................................................................................................... 44
Reference ........................................................................................................................ 45
Nomenclature

Alphabetic Letters

A  Absorbance

c  Concentration of solution

d  Optical path length

L  Length of the sample channel

m  Slope of the straight line

s_{\text{blank}}  Standard deviation of the blank signal

s_s  standard deviation of the measurement
Greek Letters

\( \varepsilon \) Absorbance coefficient

\( \theta_i \) Incident angle of light
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>APD</td>
<td>Avalanche photodetector</td>
</tr>
<tr>
<td>EWOD</td>
<td>Electrowetting-on-dielectric</td>
</tr>
<tr>
<td>LED</td>
<td>Light emitting diode</td>
</tr>
<tr>
<td>LOC</td>
<td>Lab-on-a-chip</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>PDMS</td>
<td>Poly(dimethylsiloxane)</td>
</tr>
<tr>
<td>PEB</td>
<td>Post-exposure bake</td>
</tr>
<tr>
<td>POC</td>
<td>Point-of-care</td>
</tr>
<tr>
<td>MPPC</td>
<td>Multi-pixel photon counter</td>
</tr>
</tbody>
</table>
List of Tables

Table 1. Physical properties of liquid metal, Glinstan.

Table 2. List of materials.

Table 3. Comparisons liquid metal mirros to air mirrors and linear channel.

Table 4. Comparisons to other spectrometers.
List of Figures

Figure 1. Beer-Lambert Law.

Figure 2. Efficiency of optical path extension.

Figure 3. Czerny-Turner spectrometer.

Figure 4. Chip design for optical path extension using liquid metal mirrors.

Figure 5. Chip design for spectrometric analysis.

Figure 6. Fabrication process.

Figure 7. Definition of limit of detection (LOD) and sensitivity.

Figure 8. Liquid metal mirrors type compared to linear channel and air mirrors type.

Figure 9. LOD and sensitivity as incident angles of liquid metal mirrors vary.
Figure 10. Spectrometric analysis of phenol red solution.

Figure 11. Spectrometric analysis of trypan blue solution.

Figure 12. Various applications for further works.

Figure 13. Optical path extension combined to spectrometric analysis.
Chapter 1. Introduction

1.1. Optical measurements in lab chips

Since the first introduction to soft lithography by G. M. Whitesides[1], researchers have developed lab-on-a-chip (LOC) to analyze targets related to diagnosis and prognosis such as cardiac shocks, cancers and infectious diseases from biological samples quantitatively in a recent decade[2-14]. Conventional methods for clinical chemistry consume huge resources of biological samples, time, various equipments and experts. For example, to diagnose the patients with acute myocardial infarction who has been brought to the emergency room, conventional diagnosis steps of blood separation, reagent mixture, incubation and analysis are too complicated to diagnose in time. LOC is therefore expected to substitute conventional clinical analyzers with the advantages of automation, low sample volume, low risk of contaminations and no expert users[15]. As the needs for point-of-care (POC) are growing, needs for LOCs are increasing in the biomedical fields.

For quantitative analysis of biological samples, optical measurements including fluorescence, luminescence, absorbance and reflectance are the most widely used methods. Optical signal amplification is well organized technology
and often applied to analyze small quantity of proteins. Even though researchers have integrated LOCs successfully, they still tend to use conventional optical readers because direct optical reading methods in LOCs are not believed to be reliable yet. Since conventional optical readers are composed of various optical components such as mirrors, gratings, prisms, slits, optical fibers, light sources, filters and photodetectors, it is hard to adapt them as they are into LOCs precisely. Integration and miniaturization of optical components for LOCs should be considered at the same time.

Integration of optical readers in LOCs has advantages over conventional readers. Conventional readers generally use standardized platform of microplate, and it causes sample loss and risk of contaminations while samples are handled manually from one plate to others. Since optical path length is determined by the sample volume which it is not controlled precisely by hand, conventional readers measure and calibrate the volume of the sample. If optical reader is successfully integrated into LOCs, there’s no need to calibrate sample volume because measurement occurs directly in the sample chamber that is automatically handled. Fortunately poly (dimethylsiloxane) (PDMS) which is the most widely used material for microfluidic devices has very good optical transparency, and it plays a major role in developing optical component.
integration.

Since optical components comprising conventional optical readers are a lot and too big to integrate directly into LOCs, simple and effective optical structures or miniaturization of them should be suggested. The researches on planar microlens\[^{16,17}\], hollow prism\[^{18}\], air mirror\[^{19,20}\] are good examples. The scheme of optical measurement blocks have been tried to be adapted adequately with the different needs for optical properties as the applications vary.

This paper deals with the absorbance detection from the solution which contains the biological samples to measure. Absorbance is known to be directly proportional to the product of sample concentration and optical path length by the Beer-Lambert law\[^{21}\]. Figure 1 shows the relationship between absorbance, sample concentration and optical path length. For highly sensitive absorbance detection, optical path length should be long enough. Conventional microplates have 5~10 mm of optical path length in depth direction. Ideas to enhance efficiency of the space are needed since sample volume and the size of the device become bigger as the optical path in LOC is extended straight. Using multiple internal reflections by the difference of refractive indices between air and PDMS, optical path is extended without additional material and fabrication.
process. Air mirrors have bad reflectivity, high critical angles of $45^\circ$ for total internal reflection and cause much optical loss and extends less than 1.5 times compared to the straight channel. Figure 2 shows the efficiency of optical path extension as the incident angle varies. As the angle of incidence lowers, optical path expands longer. Whether the optical path is extended along the microfluidic sample channel direction or not, there’s a high need to get images from microscope by researchers. Optical path can be changed vertically using COC-air mirrors[22] or Si mirrors[23] but their optical properties are bad in visible wavelengths.

The previous works for imaging side view of the microfluidic devices have successfully integrated solid mirrors into microfluidic devices[24]. Even though the purpose of the research was not for the absorbance detection, direct assembly of solid mirrors can be a good solution for optical path extension. In this case, the light scattered along the microfluidic channel plane cannot be focused and the vertical alignment of solid mirrors is very critical.
Figure 1. Beer-Lambert Law. The Beer-Lambert law states the relationship between the absorbance and the product of concentration of solution and optical path length. Absorbance is directly proportional to the product of them. To detect highly sensitive absorbance, optical path length should be extended in LOC.
Figure 2. Efficiency of optical path extension. (a) shows a straight microfluidic sample channel of optical path length $L$ and (b), (c) show mirror embedded channels with incident angle of $45^\circ$, $20^\circ$ and optical path length of $1.41L$, $2.92L$ respectively. As the incident angle lowers, optical path extends longer.
For highly sensitive absorbance detection, this paper proposes a new novel platform of spectrometric analysis. It is simplified and miniaturized platform compared to the conventional spectrometers. Figure 3 shows the most widely used scheme of Czerny-Turner spectrometer. In the common Czerny-Turner design, the broad band illumination source is aimed at an entrance slit. The amount of light energy available for use depends on the intensity of the source in the space defined by the slit (width * height) and the acceptance angle of the optical system. The slit is placed at the effective focus of a collimating mirror so that the light from the slit reflected from the mirror is collimated (focused at infinity). The collimated light is refracted by the prism or diffracted from the grating and then is collected by another focusing mirror which refocuses the light, now dispersed, on the exit slit. At the exit slit, the colors of the light are spread. Because each color arrives at a separate point in the exit slit plane, there are a series of images of the entrance slit focused on the plane. Because the entrance slit is finite in width, parts of nearby images overlap. The light leaving the exit slit contains the entire image of the entrance slit of the selected color plus parts of the entrance slit images of nearby colors. A rotation of the dispersing element causes the band of colors to move relative to the exit slit, so that the desired entrance slit image is centered on the exit slit. The range of
colors leaving the exit slit is a function of the width of the slits. The entrance
and exit slit widths are adjusted together.

For direct spectrometric analysis, diffraction component have been
integrated in LOC. Hollow prism uses air mirrors mentioned above and shows
low spectral resolution. Micro lens and grating assembly[25-28] is
miniaturizing setups of conventional spectrometer and has a deep sample
chamber of about 5 mm vertically which is not usual in microfluidic devices.
Optical path in the vertical direction would be worse in getting uniform optical
path length if compared to that in the microfluidic channel direction. Therefore,
reproducible absorbance analysis with high sensitivity and resolution can be
achieved if microfluidic sample channel, optical path extension and the
spectrometric analysis happen in the same planar direction.

For planar integration of optical elements such as grating, slits, mirrors and
lenses, this paper proposes liquid metal material. Table 1 shows the physical
properties of liquid metal, galinstan which is widely used for thermometer
because of low toxicity. Once it is injected into microfluidic channel, it is so
easily we on the surface of PDMS and glass, that it can be used as a good
material for the highly reflective mirror.
Figure 3. Czerny-Turner spectrometer. This is the most widely used spectrometer scheme composed of collimating mirror, focusing mirror, slits and wavelength-tunable rotating grating. For high spectral resolution, the slit is made to be narrower.
<table>
<thead>
<tr>
<th>Properties</th>
<th>Descriptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition</td>
<td>Ga 68.5 %, In 21.5 %, Sn 10 %</td>
</tr>
<tr>
<td>Toxicity</td>
<td>Low toxicity</td>
</tr>
<tr>
<td>Wetting property</td>
<td>Easy wetting on glass</td>
</tr>
<tr>
<td></td>
<td>Gallium oxide coating to prevent wetting</td>
</tr>
<tr>
<td>Boiling point</td>
<td>&gt; 1300 °C</td>
</tr>
<tr>
<td>Melting point</td>
<td>−19 °C</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>&lt; 10⁻⁸ Torr (at 500 °C)</td>
</tr>
<tr>
<td>Density</td>
<td>6.44 g/cm³</td>
</tr>
<tr>
<td>Solubility</td>
<td>Insoluble in water or organic solvents</td>
</tr>
<tr>
<td>Viscosity</td>
<td>0.0024 Pa·s (at 20 °C)</td>
</tr>
<tr>
<td>Thermal conductivity</td>
<td>16.5 W/m·K</td>
</tr>
</tbody>
</table>

Table 1. Physical properties of liquid metal, Galinstan. This is generally used for thermometer because it is very low toxic compared to the mercury. Once it is injected into microfluidic channel, it is so easily wet on the surface that it can be used as a good material for the highly reflective mirror.
1.2. Research objectives

This paper uses liquid metal mirrors for multiple internal reflections in a microfluidic device to extend the optical path length. Extended optical path length is expected to make high sensitivity and low limit of detection with low optical loss and highly effective light focusing. With various incident angles of 20°, 30°, 40° and 50°, optical path extension is effectively save the size of the lab chips.

For simple spectrometric analysis using optical fibers, a new novel platform is proposed. Liquid metal is used to make optical components such as lenses, mirrors and slits. Diffraction element of transmissive grating is fabricated using SU-8 mold and PDMS peel-off. It is integrated into microfluidic device with planar focusing lens embedded. Output spectrum is demonstrated with 3 optical fibers of 50 μm core, 150 μm cladding and 250 μm coating. They are centered to measure 400 nm, 500 nm and 600 nm respectively.
Chapter 2. Materials and methods

2.1. Chip design

Source light delivered through optical fiber has about 20° of spread angle. Ideal light source is collimated along the flow direction of sample chamber. Conventional optical setup uses collimated laser source or collimating lens. Lens has generally 3 dimensional structures for focusing, collimating or scattering. This paper uses planar lens that needs no additional material or fabrication and collimates light source. The radius of curvature of collimating planar lens is 150 μm and it can be thought to be a thin lens. The sample lens is applied again at the opposite side of optical fiber that is connected to the photodetector.

Light reflected by the first liquid metal mirror is focused at 0.5 mm apart. A trivial error caused by the small difference of refractive indices between PDMS and sample solution can be ignored. The second liquid metal mirror collimates the light again to deliver to the photodetector. The radiuses of curvature are 1.22 mm, 1.32 mm, 1.66 mm and 2.22 mm with incident angles of 20°, 30°, 40° and 50° respectively.

The gaps between sample channel and liquid metal mirror are about 100
which is relatively narrow compared to the height of the microfluidic channel, 250 μm. If sample solution and liquid metal is not handled by syringe pumps, channel walls tend to break down or the curvature is distorted easily.

The purpose of this device is to measure absorbance for analyzing the concentration of the solution. Figure 4 shows the chip design and the connections. Optical path extension is achieved longer as the incident angle lowers. 4 kinds of incident angles are applied, 20°, 30°, 40° and 50°. To focus the light better, planar lenses are integrated where the sample channel and optical fibers meet as explained above. Optical path length is designed to be 3 mm equally to know the absorbance measurements change as the incident angles change. If the incident angle does not affect the reflectance of liquid metal mirrors, optical path can be extended longer with the same size of sample chamber in a microfluidic device. Moreover, scattered light through sample chamber can be focused to enhance the signal to noise ratio.

Optical fiber cannot be reassembled to the device, but since the end of optical fiber is configured as commercial SMA block, various kinds of lasers, LEDs and photodetectors can be used at the same device. As the color of sample changes, the combination of light source and detector should change too. If sample chamber is washed, the device can be reused many times.
Figure 4. Chip design for optical path extension using liquid metal mirrors. Optical fibers are connected to light source and photodetector. Optical path length is 3 mm with various incident angles of 20°, 30°, 40° and 50°. Photodetector signal includes noise that would be calibrated with averaging method. The noise is measured to calculate the standard deviation.
The purpose of the second device is spectrometric analysis using optical components made by liquid metal channels. A new platform using liquid metal and PDMS grating measures absorbance spectrometric response. All optical components such as thin planar lens, optical fibers, slits and transmissive grating are integrated in planar direction and the liquid metal is used to make entrance slit of light. This scheme is directly adapted to the lab chips which automatically handle samples. Absorbance meter is integrated into one simple lab chip. Figure 5 shows the chip design of the spectrometer and the connections. It is composed of light source, avalanche photodetector(APD), optical fibers, sample chamber, liquid metal slit and elastomeric grating.

Elastomeric grating has 100 grooves per 1 mm. Distance between grating patterns is 10 \( \mu m \) with 30° of blaze angle. Mold is composed of SU-8 photoresistor which is the most commonly used for soft lithography. Since the height of mold is 250 \( \mu m \), aspect ratio is about 25:1. To avoid complicated fabrication process, the grating pattern of align mask is an array of rectangular blocks with width of 2 \( \mu m \) and length of 10 \( \mu m \). The gap between rectangular blocks is 10 \( \mu m \). The energy of UV exposure was 260 mJ/cm\(^2\).

Since the shape of sample chamber ends sharply, light tends to be focused at the front of the liquid metal slit. The difference of refractive indices between
sample solution and PDMS induces total internal reflection and liquid metal mirror reflects the refracted light. It is thought to be helpful to enhance the effectiveness of absorbance detection.

The gap between two liquid metal channels is 10 μm which can be reduced to 2 μm with high pressure into liquid metal channels. Since liquid metal never allows for light to pass through, it is a perfect material for optical slit. Because of sharp shape of slit, bubbles are easily generated. Too much pressure to remove bubble can destroy the wall between liquid metal channels.

Light that passed through slit is collimated by planar thin lens. If it’s not collimated, spectral resolution becomes hard to predict. Diffracted light by transmissive grating is reflected by liquid metal mirror. The purpose of this mirror is to enhance spectral resolution in a small area of device for detection by optical fibers. The radiuses of curvature of planar thin lens and liquid metal mirror is 120 μm, 110 μm respectively.
Figure 5. Chip design for spectrometric analysis. Optical fibers are connected to light source and photodetectors. Detection wavelength are centered at 400 nm, 500 nm, 600 nm respectively. Optical path length is 3 mm and the light passing liquid metal slit is collimated by planar lens and diffracted by the PDMS integrated transmissive grating of 100 grooves/mm. For higher spectral resolution, the diffracted light is reflected by liquid metal.
2.2. Fabrication process

A new device platform using liquid metal to extend optical path in microfluidic channel direction is suggested. Table 1 shows the property of liquid metal, Galinstan. It has a high reflectivity and easily wetting property. It acts as liquid in room temperature and doesn’t solid over -19°C. Incident angle doesn’t have to consider the critical angle that is convenient to extend optical path effectively and we demonstrated incident angles of 20°, 30°, 40° and 50°. For comparisons to air mirror devices, the same device can be used if liquid metal is not injected. Linear channel with the same optical path length has been used for comparison too.

The devices have been fabricated by casting of PDMS (Sylgard 184 elastomer kit, Dow Corning, Midland, MI, USA) in an SU-8 master (MicroChem, Corp., Newton, MA, USA). The technology is extremely simple and it has been deeply studied and reported, nevertheless, for completeness it is reproduced here. After informal cleaning and dehydrating a 0.5 mm thick Boron doped Si wafer at 175°C for 20 minutes, the substrate is spin-coated with 250 μm of a SU-8 layer at 2500 rpm for 30 seconds. Afterwards, the substrates are baked at 65°C and 95°C for 7 and 45 minutes respectively and exposed to UV light of 350 mJ/cm² with a mask. The post-exposure bake (PEB)
follows at 65°C and 95°C for 5 and 15 minutes respectively. The PEB was followed by developing the structures, finishing the definition of the master. The prepolymer was obtained by mixing the curing agent with the elastomer base in a 1 : 10 ratio (v : v). The resulting mixture was subsequently degassed to remove air bubbles, poured over the master and cured for 180 minutes at 80°C. Afterwards, the cured PDMS was peeled off from the master and the fluidic ports were opened. Then, both the PDMS and a second slide glass of soda lime substrate were exposed to an oxygen plasma. Immediately afterwards, optical fiber is laid on the guide line of the PDMS device and it is followed by covalent bonding causing its irreversible sealing. For accurate alignment and tight bonding, optical fiber is pulled out gently with the top side of the PDMS device pushed down. Figure 6 shows the entire fabrication process.

The height of microfluidic channel is 250 μm which is thought to be high for normal soft lithography. If the mold is shown in section, microfluidic channels for liquid metal can easily be slanted with overexposure or underexposure. That distorts the optical path in vertical direction which is not intended for planar optical extension. If the mold is fabricated with expensive and delicate process such as LIGA, that problem is thought to be solved easily.
With just simple tuning of exposure condition, no expensive lithography is needed. Si wafer scatters UV source at the bottom side of the mold and top side of mold is always exposed the most. It causes shape of mold to be a lens and focuses light to the center of vertical direction. To avoid cracks along the channel walls, PEB is done once more with low temperature, 60°C for 4 hours.
Figure 6. Fabrication process. The entire softlithography is illustrated. For accurate alignment and tight bonding between optical fibers and PDMS device, optical fiber is pulled out slowly and PDMS device is pushed down.
2.3. Experimental setup

For testing the optical path extension, fluorescence dye of fluorescein is used. Fluorescein is a fluorophore commonly used in microscopy, in a type of dye laser as the gain medium, in forensics and serology to detect latent blood stains, and in dye tracing. Fluorescein has an absorption maximum at 494 nm and emission maximum of 521 nm (in water). Fluorescein also has an isosbestic point (equal absorption for all pH values) at 460 nm. Fluorescein sodium is used extensively as a diagnostic tool in the field of ophthalmology and optometry, where topical fluorescein is used in the diagnosis of corneal abrasions, corneal ulcers and herpetic corneal infections. It is also used in rigid gas permeable contact lens fitting to evaluate the tear layer under the lens. Intravenous or oral fluorescein is used in fluorescein angiography in research and to diagnose and categorize vascular disorders in e.g. legs, including retinal disease macular degeneration, diabetic retinopathy, inflammatory intraocular conditions, and intraocular tumors, and increasingly during surgery for brain tumors. In cellular biology, the isothiocyanate derivative of fluorescein is often used to label and track cells in fluorescence microscopy applications (for example, flow cytometry). Additional biologically active molecules (such as antibodies) may also be attached to fluorescein, allowing biologists to target
the fluorophore to specific proteins or structures within cells. This application is common in yeast display. Fluorescein can also be conjugated to nucleoside triphosphates and incorporated into a probe for in situ hybridisation. Fluorescein-labelled probes can be imaged using FISH, or targeted by antibodies using immunohistochemistry. The latter is a common alternative to digoxigenin, and the two are used together for labelling two genes in one sample.

For testing the spectrometer, trypan blue and phenol red are used. Trypan blue is commonly used in microscopy (for cell counting) and in laboratory mice for assessment of tissue viability. The method cannot distinguish between necrotic and apoptotic cells. Most living tissues prosper at a near-neutral pH; that is, a pH close to 7. The pH of blood ranges from 7.35 to 7.45, for instance. When cells are grown in tissue culture, the medium in which they grow is held close to this physiological pH. A small amount of phenol red added to this growth medium will have a pink-red color under normal conditions. Typically 15 mg / 1 L is used for cell culture. In the event of problems, waste products produced by dying cells or overgrowth of contaminants will cause a change in pH, leading to a change in indicator color. For example, a culture of relatively slowly-dividing mammalian cells can be quickly overgrown by bacterial
contamination. This usually results in an acidification of the medium, turning it yellow. Many biologists find this a convenient way to rapidly check on the health of tissue cultures. In addition, the waste products produced by the mammalian cells themselves will slowly decrease the pH, gradually turning the solution orange and then yellow. This color change is an indication that even in the absence of contamination, the medium needs to be replaced (generally, this should be done before the medium has turned completely orange). Since the color of phenol red can interfere with some spectrophotometric and fluorescent assays, many types of tissue culture media are also available without phenol red.

Light source is precisely controlled by constant current driver (Thorlabs). MPPC is used for highly sensitive photo-detection. White LED is turned on with 350 mA of constant current. Bundle software provided by Hamamatsu is used to control and acquire signal from MPPC. Shutter speed and exposure time are configurable. The signal is captured for 5 seconds after 30 seconds of warming up of LED. Table 2 shows the list of materials for experimental setups.
### Table 2. List of materials.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence dye</td>
<td>Fluorescein (494-521 nm, excitation-emission, <em>Sigma Aldrich</em>)</td>
</tr>
<tr>
<td>Color dye</td>
<td>Phenol red (<em>Sigma Aldrich</em>), Trypan blue (<em>Invitrogen</em>)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Optical components</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light source</td>
<td>Power LEDs (455 nm, white, <em>Thorlabs</em>) DC2100 (High power LED driver, <em>Thorlabs</em>)</td>
</tr>
<tr>
<td>Microscope</td>
<td>Eclipse TS-100 (<em>Nikon</em>)</td>
</tr>
<tr>
<td>CCD/ Driver</td>
<td>DS-2MBWc / DS-U2 (Black and white CCD, <em>Nikon</em>)</td>
</tr>
<tr>
<td>Photodetector</td>
<td>C10507-11-025C (MPPC, <em>Hamamatsu</em>)</td>
</tr>
<tr>
<td>Liquid metal</td>
<td>Galinstan (<em>Geratherm</em>)</td>
</tr>
<tr>
<td>Fiber optics</td>
<td>AFS50/125Y (Core 50 μm, Cladding 125 μm, Coating 250 μm, <em>Thorlabs</em>)</td>
</tr>
</tbody>
</table>

Light source of power LED is precisely controlled by constant current generating driver. MPPC is multi-pixel photon counter produced by Hamamatsu, which is made up of multiple APD (avalanche photodiode) pixels operated in Geiger mode. All optical components are connected by the SMA connectors.
2.4. Evaluation criteria

The most generally accepted quantitative definition of detection limit is that it is the minimum concentration or mass of analyte that can be detected at a known confidence level. This limit depends upon the ratio of the magnitude of the analytical signal to the size of the statistical fluctuations in the blank signal. That is, unless the analytical signal is larger than the blank by some multiple k of the variation in the blank owing to random errors, it is impossible to detect the analytical signal with certainty. Thus, as the limit of detection is approached, the analytical signal and its standard deviation approach the blank signal and its standard deviation. The minimum distinguishable analytical signal is then taken as the sum of the mean blank signal plus a multiple k of the standard deviation of the blank. The resulting data are then treated statistically to obtain mean blank signal and its standard deviation. Recommended value of k is 3. Figure 7 shows the equations of LOD[29] and sensitivity[30].

The quantitative definition of sensitivity that is accepted by the International Union of Pure and Applied Chemists (IUPAC) is calibration sensitivity, which is the slope of the calibration curve at the concentration of interest. A disadvantage of analytical sensitivity is that it is often concentration dependent since standard deviation of sensor varies with concentration.
Limit of Detection (LOD)* = \frac{3 \cdot s_{\text{blank}}}{m}

Sensitivity** = \frac{m}{s_s}

\( m \): slope of the straight line
\( s_{\text{blank}} \): standard deviation of the blank signal
\( s_s \): standard deviation of the measurement

**Figure 7.** Definition of limit of detection (LOD) and sensitivity. LOD is generally used 3 times of standard deviation of blank signal divided by slope of the straight line. Sensitivities vary as the concentration of solution varies. In this paper, sensitivity means the minimum sensitivity of the measured absorbance.
Chapter 3. Results and discussions

3.1. Optical path extension

The optical path extension has been accomplished with small angle of incidence, 20° without trade-offs of sensitivity and LOD. Table 3 shows the microscope imaging comparisons liquid metal mirrors to air mirrors and linear channel. No additional device is fabricated for comparison test to air mirror. Liquid metal mirror works as air mirror if liquid metal channel is not filled with anything. Air mirror doesn’t reflect incident angles below 45° of critical angle but liquid metal mirror works good with lower optical loss. The working principle of liquid metal mirror is basically different from the air mirror which is based on the difference of refractive indices between materials. The molecular structure of liquid metal is dense like other metals in liquid status which enables reflect light with high efficiency. Generally speaking, metals reflect light with their own colors, but liquid metal, galinstan distorts the color of incident light much less than other metal materials. Since liquid metal mirror doesn’t have to consider incident angle, multiple internal reflections have been done successfully with small incident angle, 20° and smaller angle is expected to be adapted, too. If incident angle becomes lower than 5°, optical path can be
extended very longer in a narrow sample chamber. It can be used to detect single droplet to sort by the number of cells trapped. For simple comparisons, linear channel is designed to be a rectangular shape, and it would work better if it’s been a sharpening shape in outlet direction. Since that kind of design includes other design considerations, it’s not applied here.

Figure 8 shows the comparisons of LODs and sensitivities, which says the enhancements of liquid metal mirrors. It is caused by the focusing of incident light which results lower optical loss by scattering. In the concentration range under 500 nM, the absorbance measured more in detail to compare the LOD calculated and graph which match well. Even though optical loss in air mirror is relatively higher, LOD and sensitivity of air mirror is almost the same as these of linear channel. Liquid metal mirror has better performance compared to the air mirror and linear channel. With the absorbance detection using 5 μM of fluorescein, LOD becomes 4.5 times lower from 377 nM to 84 nM and sensitivity becomes 3.6 times higher from 1.29x10^{-3} to 3.62x10^{-4}.

As the incident angle lowers, optical path can be extended more effective. Various incident angles of 20°, 30°, 40° and 50° are tested and figure 9 shows there is no trade-offs of LODs and sensitivities with incident angles. It means the performance is maintained if optical path is extended with small angle of
incidence. Since incidence angle can be lowered below 20°, this feature can be adapted to various applications.

Liquid metal mirrors can be designed flexibly to focus or collimate light. For absorbance measurement in a microfluidic device, there is a strong need to minimize the size of the conventional flowcells of optical path length from several mm to several hundreds of mm. The purpose of flowcell is to detect small number of concentrations by absorbance detection in a long linear sample chamber. The sample should be delivered from other container that results sample loss and risk of contaminations. Since there’s no additional focusing element in linear shape of flowcells, sensitivity and LOD cannot be enhanced. A new novel platform of optical path extension using liquid metal mirrors consumes small sample volume directly from sample chamber in a microfluidic device, and enhances LOD and sensitivity with lower optical loss than linear channel or air mirror.
Table 3. Comparisons liquid metal mirrors to air mirrors and linear channel. Air mirrors do not reflect incident light when incident angle is below 45°, critical angle. But liquid metal mirrors reflect and focus incident light well.

<table>
<thead>
<tr>
<th>Incident angle</th>
<th>20°</th>
<th>30°</th>
<th>40°</th>
<th>50°</th>
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<tbody>
<tr>
<td><strong>Liquid metal mirror</strong></td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
</tr>
<tr>
<td><strong>Air mirror</strong></td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
</tr>
<tr>
<td><strong>Linear channel</strong></td>
<td><img src="image9" alt="Image" /></td>
<td><em>Solution: 5 μM of fluorescein&lt;br&gt;Light source: 455 nm power LED @ 350 mA</em></td>
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</table>
Figure 8. Liquid metal mirrors type compared to linear channel and air mirrors type. Liquid metal mirrors collect scattered light better causing lower LOD and higher sensitivity.
Figure 9. LOD and sensitivity as incident angles of liquid metal mirrors vary. As incident angles vary, LOD and sensitivity are maintained well. This result implies that optical path extension can be obtained effectively with small angle of incidence.
3.2. Spectrometer

The gaps between optical fibers for R,G,B measurements are 350 $\mu$m respectively. This new design of spectrometer provides a detailed spectral resolution, but it is limited by optical fiber setup. This platform is useful for the applications to measure profile of color using optical fibers. The core size of optical fibers is 50 $\mu$m, so it is the same as the 50 $\mu$m of optical fibers arranged in the gaps of 350 $\mu$m. The tests are to know the feasibility for measurements of profile of solution color and absorbance change as concentrations vary. LOD and sensitivity are calculated after measurements too.

Solutions of different colors, phenol red and trypan blue are used to demonstrate the spectrometer using liquid metal. Figure 10 shows the result of the absorbance measurement of phenol red from 3 optical fibers that are centered to measure 400 nm, 500 nm and 600 nm respectively. Then red fiber of 600 nm is plotted with 212 nM of LOD and 1.09x$10^{-3}$ of sensitivity. Figure 11 shows the result of the absorbance measurement of trypan blue from 3 optical fibers too. Then blue fiber of 400 nm is plotted with 154 nM of LOD and 8.10x$10^{-4}$ of sensitivity. For optical fiber using applications, this platform is very simple and effective for spectral analysis. As two figures show, profiles of colors from solutions of different colors express absolutely different. The
maximum absorbance has happened in optical fiber aligned to red color measuring phenol red and blue color measuring trypan blue as expected. White LED is thought to be a disadvantage to reduce the performance of LOD and sensitivity, but it proved that the spectral resolution is high enough. Profile of colors measured can be developed to know the kinds and concentrations of sample solutions.

Comparisons to previous researches are shown in table 4. This scheme is closer to the structure of conventional spectrometer and optical components of mirrors, lenses and slits are fabricated simply just by microfluidic channel and liquid metal. Hollow prism is simple for fabrication compared to the transmissive grating integration with trade-off of spectral resolution. In planar optical path extension, optical components such as optical fibers and photodetectors are essential. If optical fiber is used, simply measurement by microscope is impossible. This novel design of device performs well with simple optical configuration. This platform is available for both of concentration measurement of known solution and analyzing unknown material in solution status.
Figure 10. Spectrometric analysis of phenol red solution. Optical fiber of 600 nm wavelength centered detection shows good sensitivity and LOD.
Figure 11. Spectrometric analysis of trypan blue solution. Optical fiber of 400 nm wavelength centered detection shows good sensitivity and LOD.

<table>
<thead>
<tr>
<th>LOD</th>
<th>Sensitivity</th>
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<tr>
<td>154 nM</td>
<td>8.10 x 10^{-4}</td>
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<tr>
<td>Figure</td>
<td>Diffraction element</td>
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<tr>
<td><img src="image" alt="Transmissive PDMS grating" /></td>
<td>Transmissive PDMS grating</td>
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<tr>
<td><img src="image" alt="Hollow prism" /></td>
<td>Hollow prism</td>
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<tr>
<td><img src="image" alt="Commercial transmissive grating" /></td>
<td>Commercial transmissive grating</td>
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</table>

**Table 4.** Comparisons to other spectrometers. Hollow prism using air mirror has low reflectivity causing optical loss. Commercial transmissive grating type has unusually deep sample chamber and bad quality of collimating lens.
Chapter 4. Conclusion

4.1. Summary

Liquid metal mirrors can be designed with minimum optical loss and incident angle, which makes the size of the lab chip smaller. Conventional flowcells that are used to measure absorbance are expected to be substituted by the design of multiple internal reflections. Liquid metal can be used as optical components materials such as mirror, slit, lens. Just by simple design of microfluidic channel and liquid metal injection, optical path are manipulated to focus, scatter and be blocked. High reflectivity of liquid metal mirrors results the enhancements of LOD and sensitivity, 4.5 times and 3.6 times respectively, compared to the air mirrors and linear channel. There is no trade-offs of LOD and sensitivity as the incident angle lowers.

A new platform for spectrometer analysis on lab chips have been developed using liquid metal. By simple design of microfluidic device and liquid metal material injection, spectrometer close to conventional device scheme has been achieved with high LOD and sensitivity.
4.2. Further works

Previous works using liquid metal in a microfluidic device are about electrowetting property and applications related to liquid metal of droplet manipulations[31,32]. Liquid metal requires high potential for electrowetting and can be handled within expected range of wetting property, so handling liquid metal in a microfluidic device is not a big deal. Using this property, optical path extension in vertical direction can be made for imaging by microscopy or CCD like figure 12 (a) shows. If device is well assembled with light source and photodetector, a lab chip can be expected to be integrated with user friendly design.

Optical fiber that has been used as waveguide in this paper too purposes to carry light with minimized optical loss[33]. Since optical fibers cannot be integrated every time in restricted area, waveguide like figure 12 (b) can be made to focus light in planar directions. Just like planar lens which is now commonly used, we expect planar waveguide using liquid metal be the solution for focusing light in a microfluidic device.

Figure 12 (c) shows a good example of single droplet analysis. Single droplet can be sorted and analyzed with absorbance detection which varies with number of cells that contains. Light with small incident angle can be
captured with small sample volume, high sensitivity and low LOD without conventional long flowcells. When minimum available design of incident angle is applied, small concentration of single droplet can be analyzed without design change of sample chambers. Droplet measurement and sorting technique by optical properties are not well developed yet compared to the manipulation techniques. This idea is expected to suggest a new common platform for design of droplet detection.

This paper presented two schemes for absorbance detection which are multiple internal reflections by liquid metal mirrors and spectrometric analysis using liquid metal slit and mirrors. If they are combined in a device, figure 13 is a good example. As the test reports of liquid metal mirrors say here, optical properties such as LOD and sensitivity are expected to be enhanced a lot if two of them are combined in a microfluidic device.

Liquid metal as a new material for optical components to integrate in a lab chip can be applied to many other applications. Absorbance and other optical properties can be found to be enhanced with developments of liquid metal components.
Figure 12. Various applications for further works. (a) shows the optical path extension in vertical direction using electrowetting property of liquid metal. (b) shows focusing light with minimum optical loss in planar direction. (c) shows multiple internal reflections with small incident angle to detect single droplet.
Figure 13. Optical path extension combined to spectrometric analysis. (a) shows the scheme for transmissive grating and (b) is for reflective grating.
Summary in Korean

본 논문에서 미세유체소자 내 액체금속거울을 이용한 광경로 확장(optical path extension)과 분광학적 분석(spectrometric analysis)을 할 수 있는 새로운 형태의 디바이스에 대한 연구를 수행하였다. 액체금속거울은 LED로부터 발광된 빛을 높은 반사율로 소량의 시료로부터 충분한 광량을 집적할 수 있다. 광경로 확장을 위한 디바이스는 20°, 30°, 40°, 50°의 다양한 각도의 입사각에 대하여 실험을 수행하였다. 선행 연구인 공기거울방식과 일자채널의 모양과 성능을 비교하였고, 성능 비교의 지표로 최소감지범위(Limit of detection, LOD)와 감도(sensitivity)를 비교하였다. 액체금속거울을 이용한 디바이스는 입사각이 작아지더라도 최소감지범위 및 감도의 손실이 발생하지 않았고, 증류수에 희석한 fluorescein의 흡광도를 측정하였을 때 입사각이 50°를 기준으로 최소감지범위는 84 nM, 감도는 3.62x10⁻⁴를 얻을 수 있었 다. 또 다른 디바이스인 스펙트로미터는 미소유체소자의 시료채널 평면 방향에서 광경로를 조작하여 광화이버로 측정을 할 수 있는 새로운 플랫폼이다. 생화학시료의 분석에 일반적으로 많이 쓰이는 phenol red와 trypan blue를 이용하여 스펙트럼분석을 수행하였는데, 최소감지범위와 감도가 각각 212 nM, 154 nM, 1.09x10⁻³, 8.10x10⁻⁴으로 정밀한 분석을 수행할 수 있음을 증명하였다.
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