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Ph.D. Thesis

**Development of Image-Based Machine Learning
Systems for Predicting Solid Mixture Ratios and
Application to Analysis of Solid-State Reactions**

(画像機械学習による混合比予測システムの開発と固相反応分析への応用)

*Graduate School of Chemical Sciences and Engineering,
Hokkaido University*

Hayato Shirakura

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Chapter 1

General Introduction

1.1. Research Background

Analysis is essential as a method for understanding existence and properties of a target substance in chemical research, like confirming target compounds in chemical reactions, detecting trace contaminants in samples, and evaluating performance of functional materials. Quantitative and qualitative analysis is based on obtaining stoichiometric ratios. (e.g. chemical reaction yield, host : guest ratio for complex, reaction equilibrium constant, etc.) In general, analytical instruments such as NMR, HPLC, GC-MS, and ICP-MS are required for analysis.¹⁻³ Although these instrumental analyses are the most accurate and reliable, sometimes have inconvenience of using expensive, large, time-consuming analytical equipment each time. When analyzing a large number of samples in a short time or screening promising reaction conditions by trial and error, it is more valuable to know approximate analytical values quickly than to obtain accurate analytical values. For such rapid analysis, molecular sensors are sometimes used.⁴ Molecular sensors are used to detect fingerprints,^{5,6} enantiomeric ratios,^{7,8} trace metal ions,^{9,10} and so on (**Figure 1-1**). These molecular sensors can detect presence and properties of species by naked eye like litmus and pH test papers through visual changes associated with chemical reactions such as luminescence.

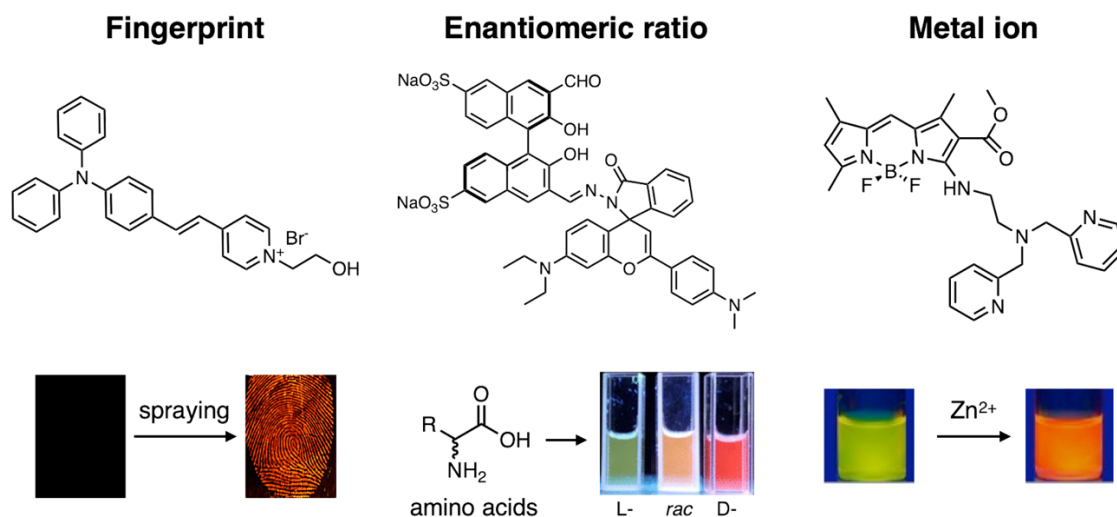


Figure 1-1. Examples of molecule sensors as rapid detection method.

Our laboratory reported synthesis of aliphatic polycarbonyl compound (**Figure 1-2**).¹¹ These polycarbonyl compound has 3,3-dimethyl-2,4-pentanedione as repeating unit, and isolated as stable compound with target chain length by prevent intramolecular cyclization and tautomerization. Polycarbonyl compound can be converted into a variety of functional molecules, like solid fluorescent chromophore and acid-resistant bulky diol using flexibility of carbon main chain.^{12,13}

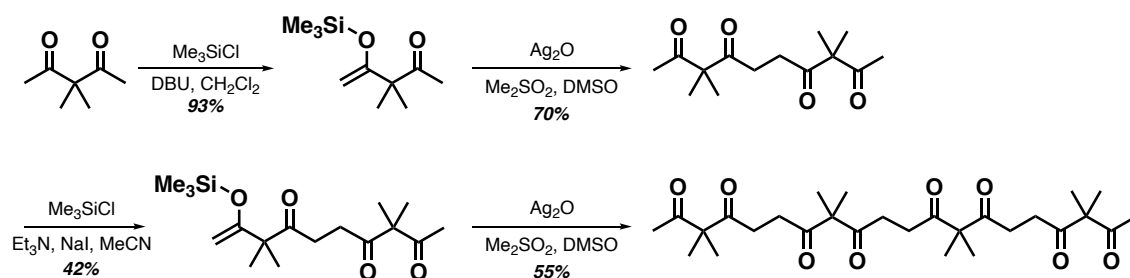


Figure 1-2. Synthesis of aliphatic polycarbonyl compounds.

Diketone moiety can be converted to isopyrazole ring by condensation reaction with $\text{H}_2\text{NNH}_2 \cdot \text{H}_2\text{O}$ (**Figure 1-3**).^{11,14} Isopyrazole ring can coordinate with various metal ions such as Ni^{2+} and Pd^{2+} , to form stable complexes (**Figure 1-4**).^{11,15}

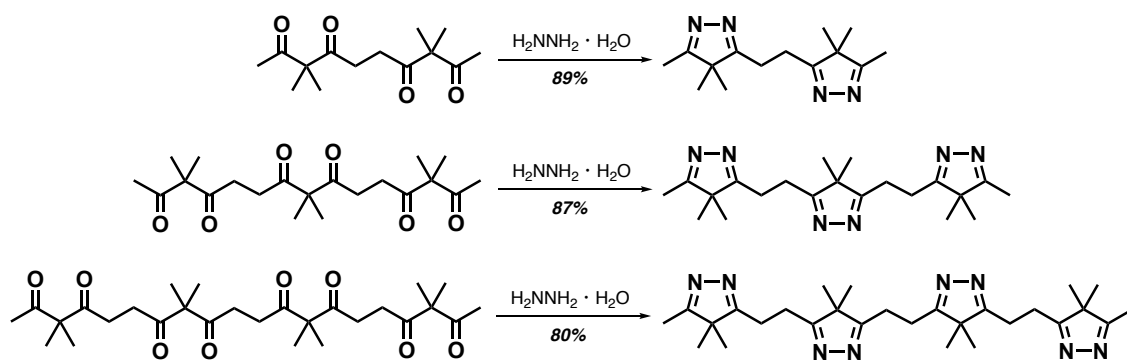


Figure 1-3. Synthesis of aliphatic isopyrazole compounds.

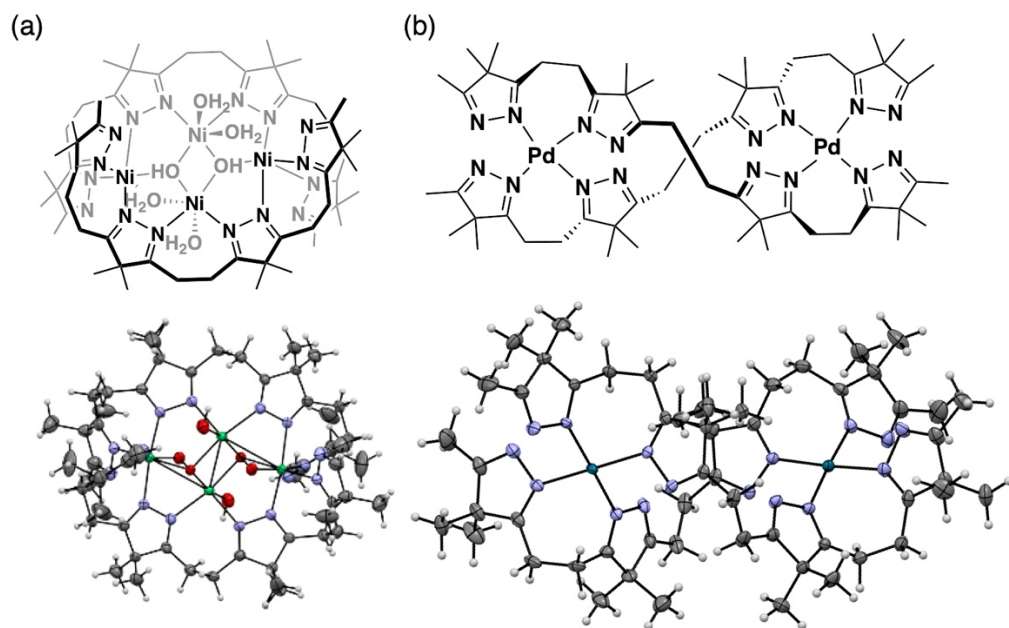


Figure 1-4. Structures of complex between aliphatic polyisopyrazole compound and (a) Ni^{2+} ion (b) Pd^{2+} ion.

Aliphatic isopyrazole can be converted to π -conjugated compounds by oxidation of *p*-chloranil (**Figure 1-5**). UV-vis spectroscopy revealed that these compounds can be used as organic chromophores by strong absorption bands in visible light region as π -conjugation becomes longer (**Figure 1-6**).¹⁴ On the other hand, aliphatic isopyrazole showed no absorption bands in visible light region (above 300 nm).

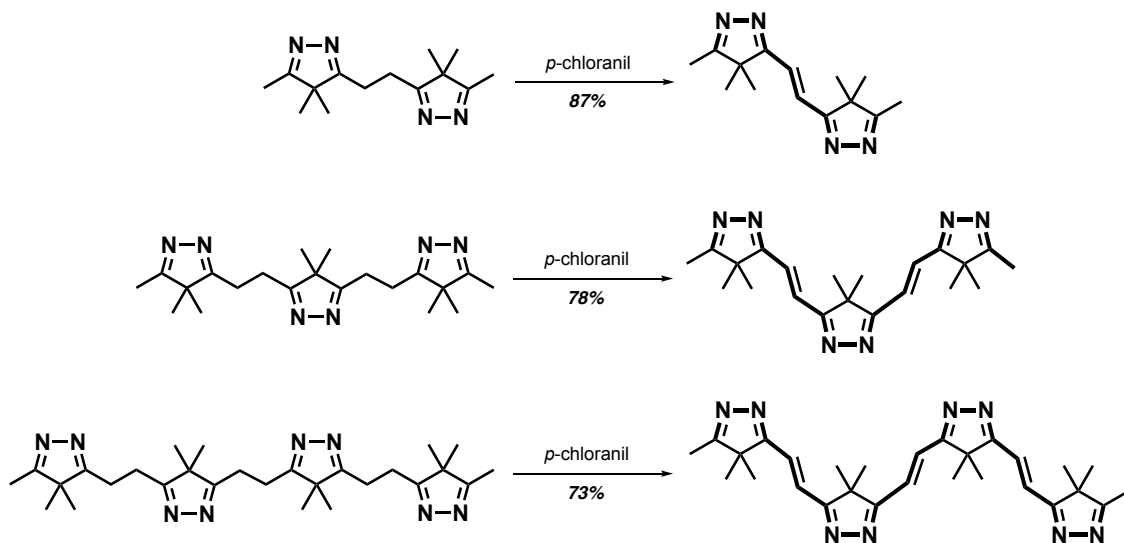


Figure 1-5. Synthesis of π -conjugated isopyrazole compounds.

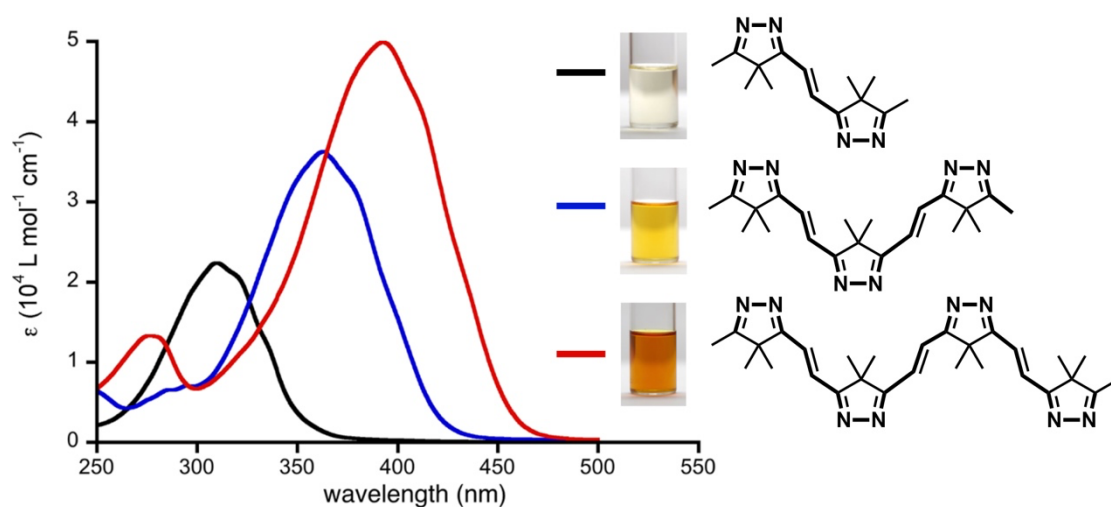


Figure 1-6. UV-vis absorption spectra of π -conjugated isopyrazole compounds (solvent : CH_2Cl_2).

Complexation ability and π -conjugation of isopyrazole ring are expected to be apply as molecular ion sensors that can detect presence of trace metal ions as color change due to complexation.

However, if appearance change of obtained molecular sensor is difficult to distinguish with naked eye, this sensor is difficult to practical application. Generally, improvement of molecular sensor was conduct by changing molecular structure. However, refinement of molecular design may result in loss of features and advantages for original molecule. This is a common obstacle in the development of molecular sensors.

The author thought that image-based machine learning can lead molecular sensor to practical application to improve detection ability than that of naked eye, instead of changing molecular structure. Machine learning achieves various predictions by construct relationships between complex parameters. Machine learning can also analyze from image data and may be able to identify subtle changes that are difficult for human eye and make predictions from visual information. For example, by analyzing image of pH test paper taken by smartphone camera, pH value can predict objectively even for difficult case to judge by naked eye.¹⁶ Therefore, I thought that improving performance of molecular sensors could be achieved by construct relationship between the appearance of compounds and their analytical values for image-based machine learning.

1.2. Applications of Machine Learning in Chemical Research

Machine learning (ML) is a subfield of artificial intelligence (AI).^{17,18} ML is put to practical use to recognize objects and character from images (recognition),¹⁹⁻²² automatically summarize text and label keywords (topic models),²³ and provide products and videos that customers might be interested in (recommendation system using reinforcement learning).²⁴ ML can be divided into three main categories: supervised learning, unsupervised learning, and reinforcement learning (**Figure 1-7**).²⁵ Supervised learning is a method in which training data is trained with “correct” data for prediction. There are two types of supervised learning: classification and regression. Classification classifies data into categories defined on data set according to features of data (for example, identify dog or cat from image data). Regression predicts or concludes other features of data based on some of available features (for example, explain relationship between parameters A and B). Various predictions are achieved by using these methods for different purposes.

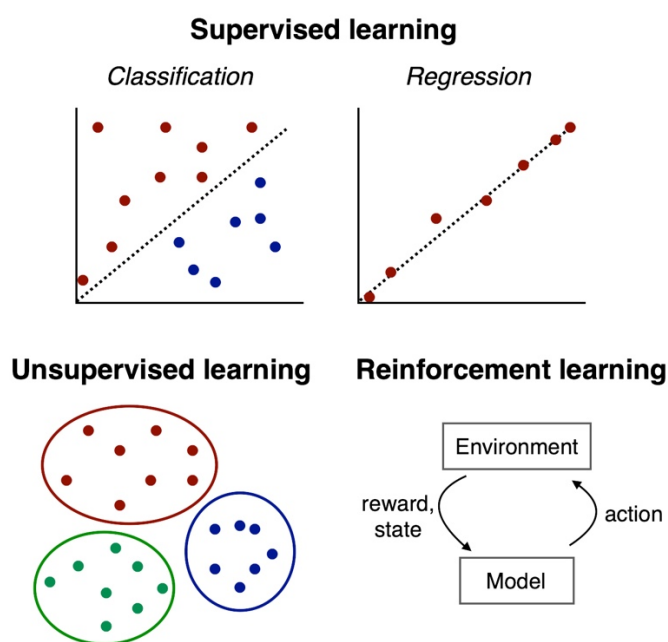


Figure 1-7. Types of approaches in ML.

One famous definition of ML is “Field of computer science that gives computers the ability to learn without being explicitly programmed” from the work of Arthur Samuel, who proposed term of “machine learning”.^{26,27} History of ML goes back to artificial neural networks that imitate mechanisms of neurons in human brain.²⁸ Artificial neural networks such as Hopfield networks and Boltzmann machines (The Nobel Prize in

Physics 2024) are the basis for principles of ML in modern era.²⁹⁻³² These days, ML has been widely used known as “3rd AI Boom”, due to increased performance and miniaturization of computers, improvement of information gathering capabilities, and economic demands associated with spread of internet connections.³³

ML has also applied in chemistry research, to predict various parameters from complex conditions such as reaction yield,³⁴ cavity size of Metal-Organic Frameworks (MOF),³⁵ pK_a value of acids,³⁶ bond dissociation energies,³⁷ reaction mechanism classification,³⁸ and so on. ML can also suggest promising experimental conditions for target compounds or materials with desired properties.³⁹⁻⁴¹ These ML predictions are contributing to faster and more efficient research (**Figure 1-8**). Moreover, ML can improve detection limit of trace metal ion from spectrum without change structure of the fluorescent molecular sensor itself.⁴²

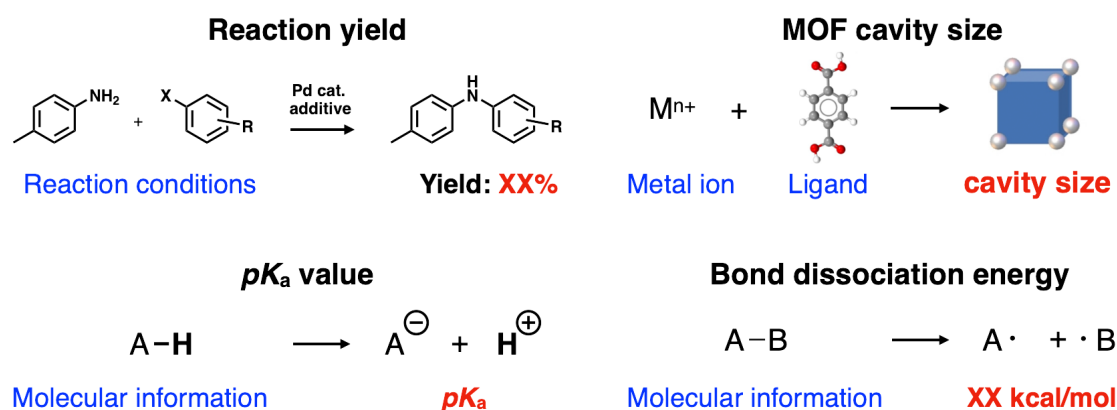


Figure 1-8. Examples of ML applications in chemical research.

For ML, predictions are made by finding relationships between quantitative variables called “feature value”. Such relationship between multiple data is called “model”. ML system can only handle numerical data. For example, for reaction yield prediction,³⁴ ML system cannot directly handle chemical equation. Instead, input feature values that represent target reaction conditions, such as ^{13}C NMR chemical shift of additives, LUMO of additives, base electronegativity, and so on. Such feature values that serve as basis of predictions are called “explanatory variables” (**Figure 1-9a**). On the other hand, feature values that target of prediction are called “objective variables”. In this case, reaction yield is the objective variable. In the case of predict inner diameter size of MOF,³⁵ physical property values related to molecules consists of MOF are the explanatory variables, and diameter sizes are objective variables (**Figure 1-9b**).

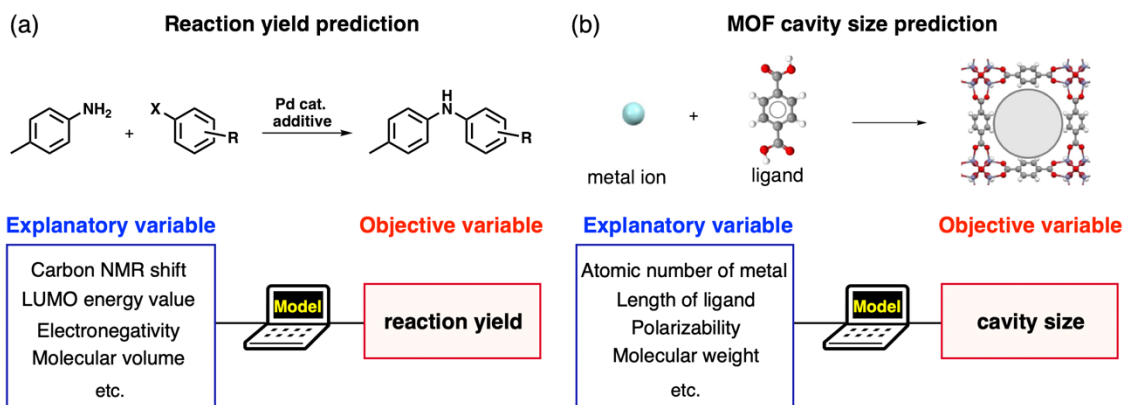


Figure 1-9. Examples of explanatory variables and objective variables to be input to construct model in supervised ML.

If an appropriate model is constructed between explanatory variables and objective variables, accurate predictions can be achieved. Appropriate combination of explanatory variables varies depending on machine learnings. Image data can also be subject to ML analysis by extract feature values.¹⁹⁻²² Even if chemically relationship or principles between variables are not clear, highly accurate predictions are possible only if an appropriate model is constructed.

1.3. Hypothesis: Relationships between Appearance and Analytical Values

In chemical research, experienced scientists can sometimes predict contents of compound and progress of chemical reaction from appearance of mixture. When optimized reaction conditions, scientists often conduct same reactions and analyze obtained products repeatedly. Of course, analysis cannot be done just by see obtained products alone. As mentioned in Section 1.1., instrumental analysis is required to obtain information, because it is impossible to see molecules directly with naked eye. However, experimenters almost always have opportunity to see reaction products with their eyes before analysis. When experimenters see the reaction products and know their analytical values repeatedly, sometimes they success to predict the results of an analysis just by looking at reaction mixture (**Figure 1-10a**). Such visual observation has the advantage that it can be performed instantly and non-destructively.

Despite these usefulness, visual observations have three main drawbacks. First, accuracy of visual observations is strongly depending on the observer's knowledge and experience. Inexperienced chemists tend to make less accurate predictions than experienced chemists. In addition, even experienced chemists occasionally make wrong or inappropriate predictions due to their physical condition, lack of concentration, and the influence of the experiment environment. It is practically impossible to make accurate judgments for hundreds or thousands of samples consecutively. Moreover, sharing information to write down decision criteria and express them objectively is quite difficult.

Image-based ML might overcome these drawbacks to digitized parameters related to scientist's knowledge and experience. Predicting analytical values from the appearance of compounds through multiple experiences is similar in some ways to the process for construct ML models. If predictions can be made from appearance of compounds, there is some kind of relationship might be existed between the appearance and its analytical value (**Figure 1-10b**). In other words, if ML model can construct the relationship between sample's appearance and its analytical value, objective predictions will be possible.

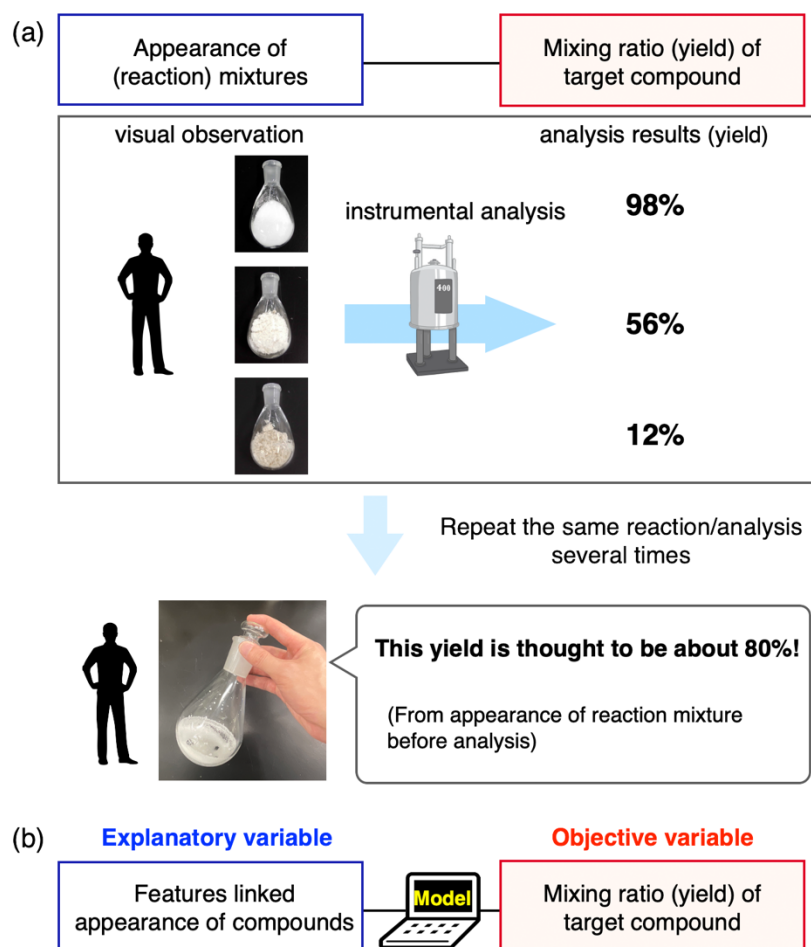


Figure 1-10. Conceptual diagram of focus and goals of this research. (a) The process of developing experience and intuition through chemical research. (b) Image-based ML prediction based on experience and intuition.

Historically, ML system was developed from experiment of programs that mimicked the workings of human brains. Since the 1970s, ML predictions system for main reaction products and retrosynthetic pathways was reported.^{43,44} This ML system recognizes all reactive sites of starting materials and automatically lists possible products, based on information extracted from chemical reaction database from academic reports.⁴⁵⁻⁴⁷ Neural network can predict which reaction is most likely to occur by classifying the similarity between types of organic chemical reactions.⁴⁸ This ML system also predict selectivity of substrates that had not reported in literatures. Predicted selectivity were consistent with actual experimental results.⁴⁹ These predictions are made based on experience and intuition by repeatedly performing and learning similar reactions (= acquiring knowledge) and classifying them appropriately (= making inferences based on perception), just like researchers do.

There have been several applications of image-based ML in chemical research.⁵⁰ However, most of these studies have focused on micro-scale images such as scanning probe microscopy (SPM) or transmission electron microscopy (TEM).^{51,52} There are still few studies that apply macro-scale images that can observe shape and color of the entire solid samples.⁵³ This is because extremely difficult to objectively describe visual information. For example, when describing colors or shapes in words, interpretations and expressions are vary from person to person, making it difficult to share the same criteria. Hence, visual information has been overlooked as a basis for prediction. Therefore, chemical predictions based on visual information would be worth trying. ML has potential to handle a huge number of samples continuously with constant accuracy that are difficult to process not only with the human eye, but also for instrumental analysis.

1.4. Overview of Thesis

The purpose of this paper is to apply image-based ML to chemical research with the goal of quantifying chemical reactions and improving performance of molecular sensor. The author synthesized trace metal ion sensors as applications of isopyrazole rings metal coordination ability derived from aliphatic polycarbonyl compounds. In the process of synthesis and analysis of these compounds, I thought image-based ML have possibility for quick and efficient preliminary analysis and expanding application limits of molecular sensor that generally depends on naked eye. To construct image-based ML system, began to verify whether ML can predict mixing ratios of solid compounds that looks different from each other. After several optimizations, ML system can also predict various mixing ratios and reaction yields for solid-state chemical reaction. Finally, attempted to chemical research applications to predict chirality from trace amount of solids and improve performance of developed metal ion sensor.

In Chapter 2, synthesized and evaluated isopyrazole metal ion sensor, and come up with an idea to introduce image-based ML as performance improvements. Compounds with metal ion coordination sites and chromophores linked by π -conjugation system are used as metal ion sensors. I synthesized BF_2 complex have strong metal coordination site and fluorescent chromophore for isopyrazole ring via π -conjugation. Yellow-green fluorescence of BF_2 complex changed to yellow in the presence of Zn^{2+} ion, while fluorescence was quenched in the presence of Cu^{2+} , Ni^{2+} , and Co^{2+} ions. Similar fluorescence change can be observed in solid state even μg scale of metal ions. So, this compound can detect and distinguish trace metal ions from solid-state fluorescence. However, detection limit of metal ions was about 1 μg , because visual changes are difficult to distinguish clearly with naked eye. I thought it may be possible to detect sub- μg scale using image-based ML, because spectral changes can be observed at even smaller equivalent of metal ion.

In Chapter 3, applied image-based ML prediction as solid chemical-compound mixing ratios and reaction yields. I first attempted to predict weight ratio of commercially available sugar and dietary salt as a model case. To improve prediction accuracy, examined appropriate methods for collecting training data (crystal arrangements and acquiring methods for image data) and ML techniques (algorithms for model and amount of training data). Sugar/dietary salt weight ratio could be predicted from image data with an average error of 4%. This prediction achieves continuously, faster, and more accurate

than human. Predictions from mobile-camera images were also successful. Mixing ratios for crystal polymorph, enantiomeric ratios, and multi-component mixtures can be predicted by same ML methods. For solid-state chemical reactions, ML model construct by images of reaction mixtures and ^1H NMR yields can predict yields with an average error of 6%. Using reaction time-lapse video, succeeded in construct ML model from a small number of experiments.

In Chapter 4, show examples for chemical research application by rapid chirality prediction and improvement of isopyrazole metal ion sensor. Enantiomeric excess of 1,1'-binaphthyl was predicted from mg-scale solids within average prediction error of 10%. Amount of Zn^{2+} ion can predict by combination of isopyrazole metal ion sensor and image-based ML. Detection of quantitation of sub- μg metal ion was succeeded, which are difficult to identify by naked eye. Image-based ML has potential not only make chemical research more efficient, but also contribute to support ability of functional materials.

In Chapter 5, the thesis is summarized, and future perspectives described. This thesis shows some examples for image-based ML as potential of objective analytical tool. This system is expected to predict various parameters related to compounds more easily by combined automation technologies of chemical reaction.

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Chapter 2

Inspiration for Image-Based Machine Learning System: Synthesis of Metal Ion Sensor Molecules

2.1. Introduction

Metal ion sensors are useful for detecting presence and kinds of metal ions that potentially harmful for human body and environments. Fluorescence sensor has advantageous to detect metal ions by naked eye through change of luminescence behavior by interactions with metal ion.¹ Organic compounds with metal ion coordination sites and chromophores (mostly boron fluorophores such as BODIPY) linked via π -conjugation are used as ion sensors (**Figure 2-1**).²⁻⁴

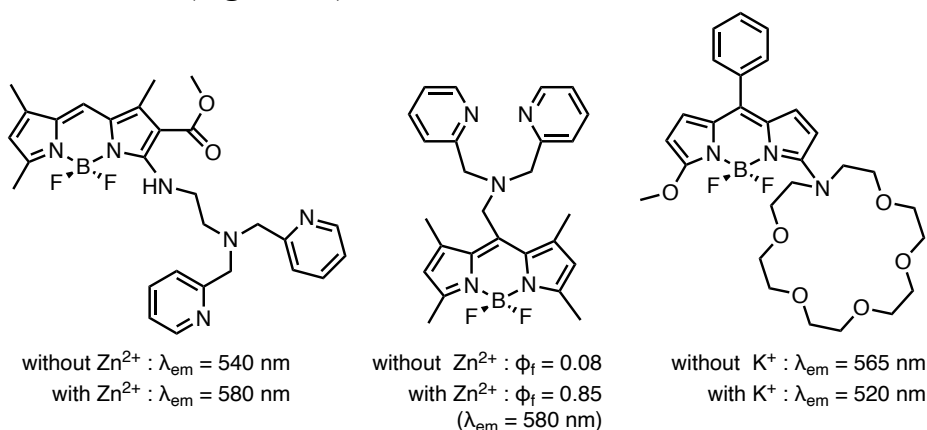


Figure 2-1. Examples of fluorescent metal ion sensors with boron fluorophore and metal ion coordination site.

As shown in Chapter 1, isopyrazole ring have ability of coordinating with metal ions. Insoluble π -conjugated polyisopyrazole compound can adsorb more than 90 % of Ni^{2+} , Pd^{2+} , Pt^{2+} ions from 10 μM solution only by mixing and filtration.⁵ Isopyrazole structure can also coordinate with various metal ions such as Cu^{2+} , Zn^{2+} and Cd^{2+} ions.^{6,7} In this chapter, to apply π -conjugated isopyrazole compound to metal ion sensor, synthesized BF_2 complex **1** that installed strong metal coordination site and fluorescent chromophore for isopyrazole ring via π -conjugation (**Figure 2-2**).

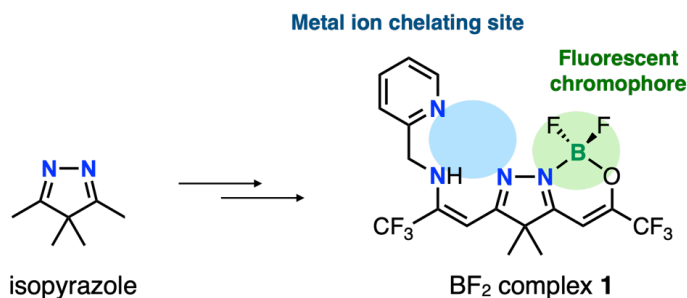


Figure 2-2. Structure and molecular design of BF_2 complex **1**.

2.2. Synthesis of BF₂ Isopyrazole Complex for Metal Ion Sensor

Synthesis BF₂ complex **1** was done by introduce functional groups for tetraketone derivative. Isopyrazole compound **2** was obtained from 3,3-dimethyl-2,4-pentanedione to obtain by 97% yield. Compound **2** was reacted with 3 equivalents of lithium diisopropylamide and then 2.3 equivalents of ethyl trifluoroacetate to obtain tetraketone derivative **3**, which inner carbonyl group is converted to isopyrazole moiety (**Figure 2-3**).

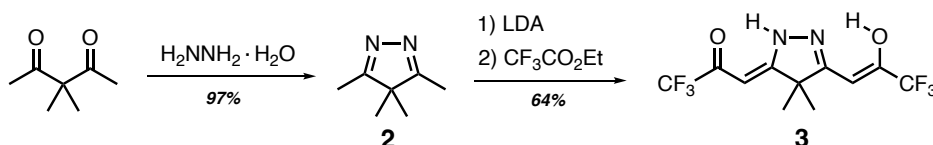


Figure 2-3. Synthesis scheme of tetraketone derivative **3**.

Tetraketone derivative **3** have six possible tautomers by keto-enol and imine-enamine tautomerization. Relative energies of tautomers in CHCl₃ were calculated by quantum chemical calculations (**Figure 2-4**).⁸ The most stable structure was isomer **3d**, which has keto-enamine-imine-enol 12 π -conjugated system. This most stable structure **3d** is consistent with the ¹H NMR spectrum, and have 16.0 kJ/mol energy difference with the next lowest energy tautomer **3c**. Therefore, compound **3** is considered to exist as a single tautomer **3d** in CHCl₃ solution. Furthermore, only the structure of **3d** with a 12 π -conjugated system was observed from single crystal X-ray structure analysis of compound **3** based on bond length, suggesting that it exists as single tautomer also in solid state (See **Figure 2-14a** in Section 2.8.).

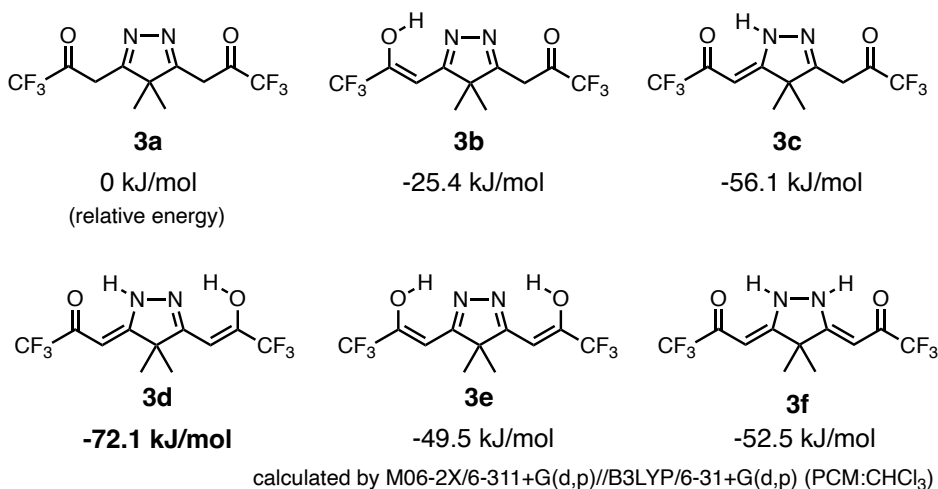


Figure 2-4. Six possible tautomers of compound **3** and relative energies calculated by quantum chemical calculations.

Reacted with 1.5 equivalents of 2-picolyamine as a metal chelating site for compound **3**, compound **4** was selectively obtained with only one of the two carbonyl groups was reacted in 58% yield. Only trace amounts of compound in which addition reaction occurred at both carbonyl groups was detected, which is thought to be because the electron density was reduced by the formation of ketimines, making it less susceptible to further addition reactions. In fact, imine addition reactions to two carbonyl groups via a π -conjugated system require more rigorous conditions.⁹ BF₂ complex **1** was obtained by react compound **4** with BF₃·OEt₂ in 83% yield (**Figure 2-5**). Boron atom was introduced only at the sterically vacant two-coordinate chelating site. Single crystal X-ray structural analysis revealed that compound **1** has a 12 π -conjugated structure (See **Figure 2-14b** in Section 2.8.).

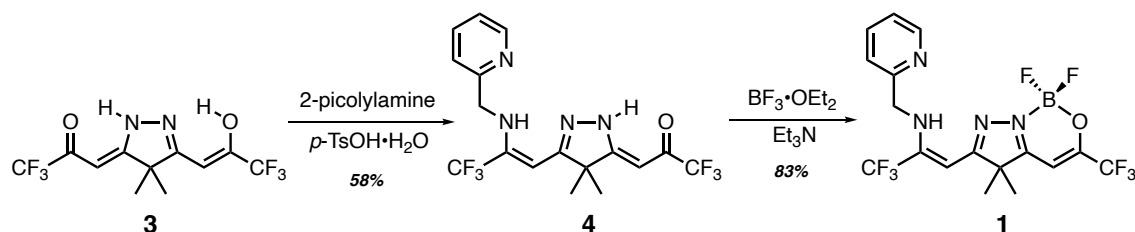


Figure 2-5. Synthesis scheme of BF₂ complex **1**.

2.3. UV-Vis Absorption and Fluorescence Emission Spectra

BF₂ complex **1** exhibit pale yellow absorption peak at 418 nm and green fluorescence peak at 503 nm in CH₂Cl₂ (**Figure 2-6**), respectively. Fluorescence quantum yield and lifetime of BF₂ complex **1** in CH₂Cl₂ are 8% and 0.8 ns, but improved to 56% and 7.4 ns in solid state, respectively (See **Figure 2-15** in Section 2.8.). This result means that complex **1** exhibit stronger fluorescence in solid state. Compounds **3** and **4** did not show any fluorescence both in CH₂Cl₂ or solid state.

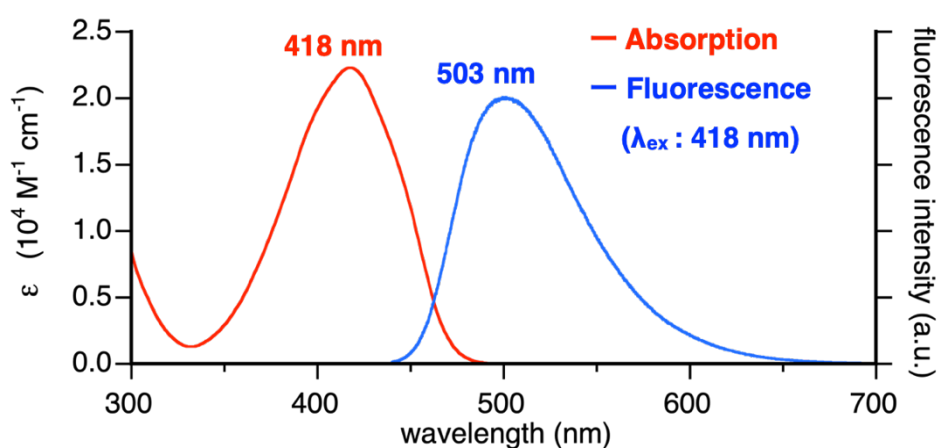


Figure 2-6. Absorption and fluorescence spectra of BF₂ complex **1** (in CH₂Cl₂).

2.4. Metal Ion Sensor Screening Test and Titration Experiments

The screening test was performed by the addition of metal ions (Zn^{2+} , Cu^{2+} , Ni^{2+} , Co^{2+} , and Fe^{2+}) corresponding to 10 equivalents of a solution (8.3 μM) of BF_2 complex **1** in methanol (**Figure 2-7**). Under room light conditions, slight yellow-green or red color changes were observed with the addition of metal ions, but it was very difficult to judge with the naked eye. Under UV light irradiation at 365 nm, the green fluorescent solution changed to yellow with the addition of Zn^{2+} , while the disappearance of fluorescence was confirmed for Cu^{2+} , Ni^{2+} and Co^{2+} . No change in fluorescence color was observed for Fe^{2+} . It is also very difficult to distinguish between solutions displaying similar colors.

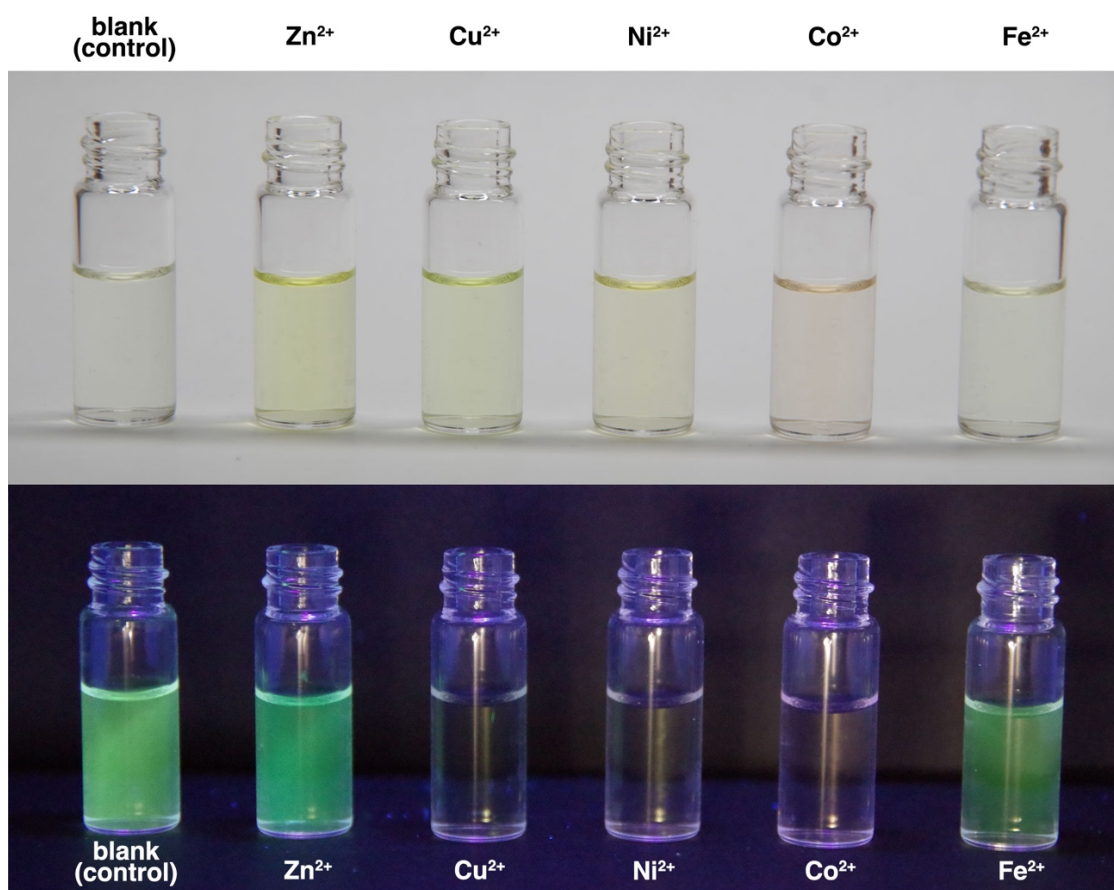


Figure 2-7. Photographs of methanol solutions of BF_2 complex **1** with (from left to right) 10 equivalent of without metal salt (control), $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$, $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$, $\text{Ni}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$, $\text{Co}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$, and $\text{Fe}(\text{OAc})_2$ (top: under ambient light, bottom: under 365 nm UV light).

Change in fluorescence by presence of Zn^{2+} ion was also confirmed by UV-vis and fluorescence spectroscopy. When $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ (0-4 equivalents) was added to a 35 μM ($\text{CH}_2\text{Cl}_2/\text{MeOH}=1:1$, v/v) solution of BF_2 complex **1**, spectral changes were clearly observed (**Figure 2-8a**). Fluorescence spectrum also showed an increase in spectral peak intensity with increasing equivalents, and in a 1.0 μM solution, approximately 100 equivalents of $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ were required for saturation (**Figure 2-8b**). As shown in **Figure 2-9**, the maximum absorption for Fe^{2+} ions does not show any significant change, while the absorption intensity of Co^{2+} , Ni^{2+} , and Cu^{2+} ions increases in the region of 450-500 nm. Therefore, it does not function as a metal ion sensor for Fe^{2+} . Co^{2+} ion showed similar quenching to that of Ni^{2+} in the range of 10 to 100 equivalents, complete fluorescence quenching was observed only with 10 equivalents of Cu^{2+} ions, showing rather high sensitivity of **1** for Cu^{2+} ion over other metal ions (**Figure 2-10**). The competition experiment using fluorescence spectroscopy revealed that ligand **1** can detect Cu^{2+} ion at 0.5–10 μM concentrations even in the presence of 30 equivalents of Zn^{2+} ions. In order to carry out detailed evaluation of metal ion sensors as described above, it is necessary to spend a lot of time and effort such as in titration experiments.

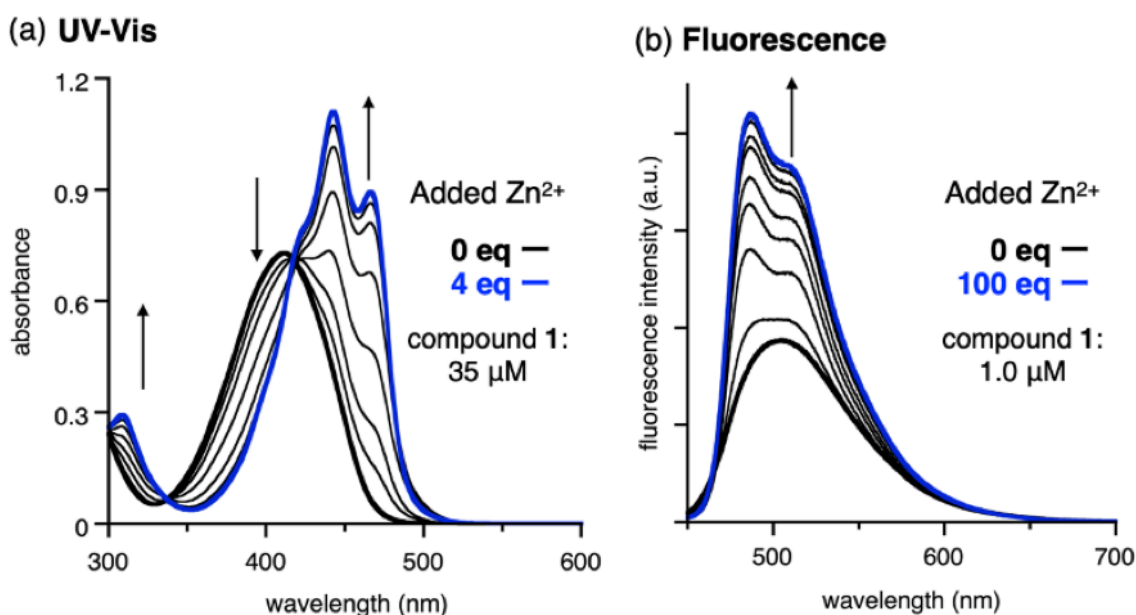


Figure 2-8. $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ titration experiment of **1** (solvent: $\text{CH}_2\text{Cl}_2/\text{MeOH}=1:1$, v/v). (a) UV-vis absorption (**1**: 35 μM) and (b) Fluorescence spectra (**1**: 1.0 μM , $\lambda_{\text{ex}} = 418$ nm).

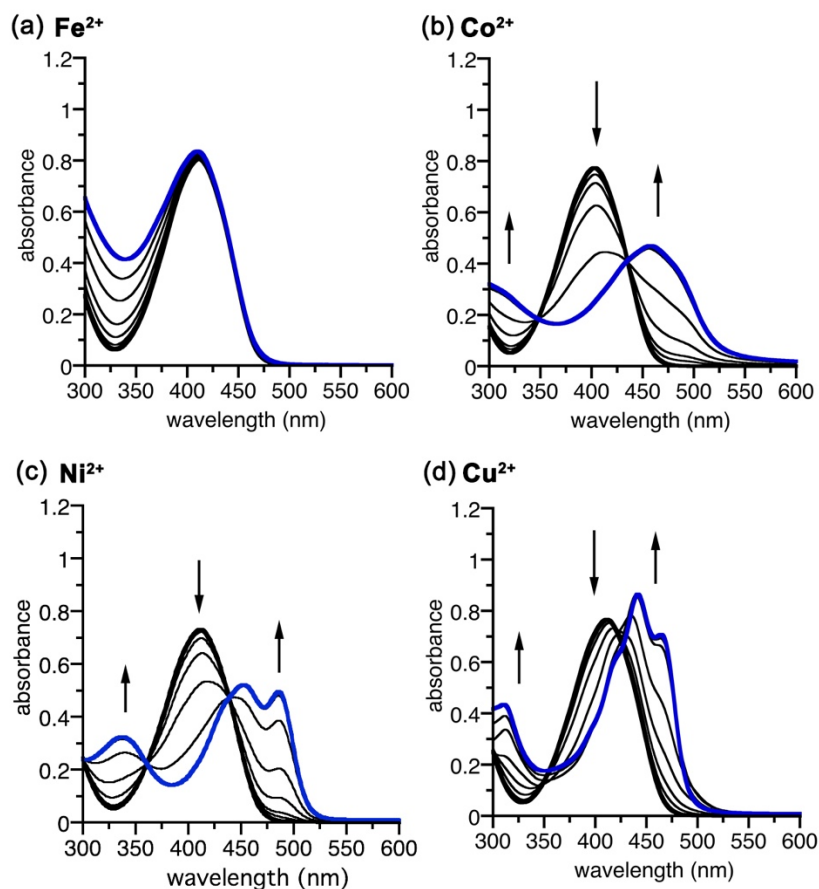


Figure 2-9. UV-vis absorption spectra of **1** (35 μM) during addition of **0** (black bold line), 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, and 4.0 equivalents (blue bold line) of (a) $\text{Fe}(\text{OAc})_2$, (b) $\text{Co}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$, (c) $\text{Ni}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$, and (d) $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (v/v, 1:1).

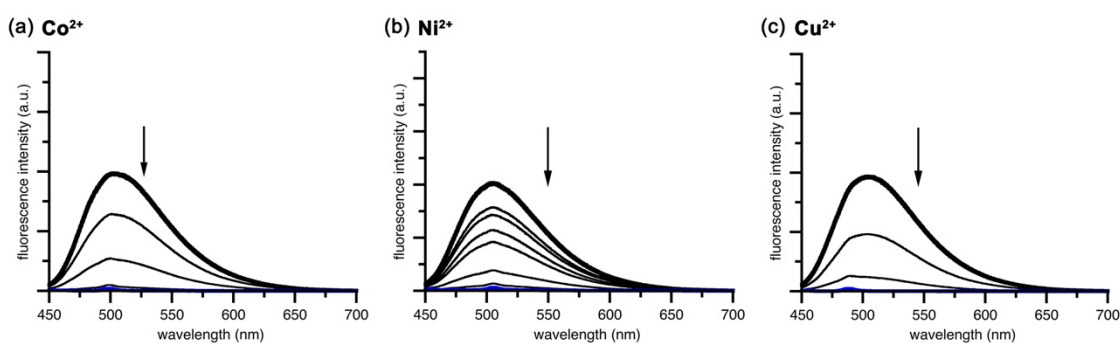


Figure 2-10. Fluorescence spectra obtained during titration of **1** (1.0 μM) with **0** (black bold line), 10, 20, 30, 40, 60, 80, and 100 equivalents (blue bold line) of (a) $\text{Co}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$ ($\lambda_{\text{ex}} = 435 \text{ nm}$), (b) $\text{Ni}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$ ($\lambda_{\text{ex}} = 439 \text{ nm}$), and with **0** (black bold line), 1, 2, 3, 4, 6, 8, and 10 equivalents (blue bold line) of (c) $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ ($\lambda_{\text{ex}} = 427 \text{ nm}$) in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (v/v, 1:1).

2.5. Metal Ion Detection by Droplet Method

I used the emission and quantitative metal complexation properties of **1** for microgram-scale metal-ion detection. A solution drop (20 μL) containing **1** (1.0 μg) was mixed with various metal acetate salts (3.0 μg), and solution and emission color changes were investigated. Solution color changes were too slight to be distinguished by the naked eyes, and fluorescence emission behavior varied depending on metal ion (**Figure 2-11**). Fluorescence from a solution drop containing Zn^{2+} ion turned slightly yellowish green as compared to **1** (blank). Solution drops containing Cu^{2+} , Ni^{2+} , and Co^{2+} ions gave fluorescence quenching, but strong emission from uncomplexed **1** was still observable in each case.

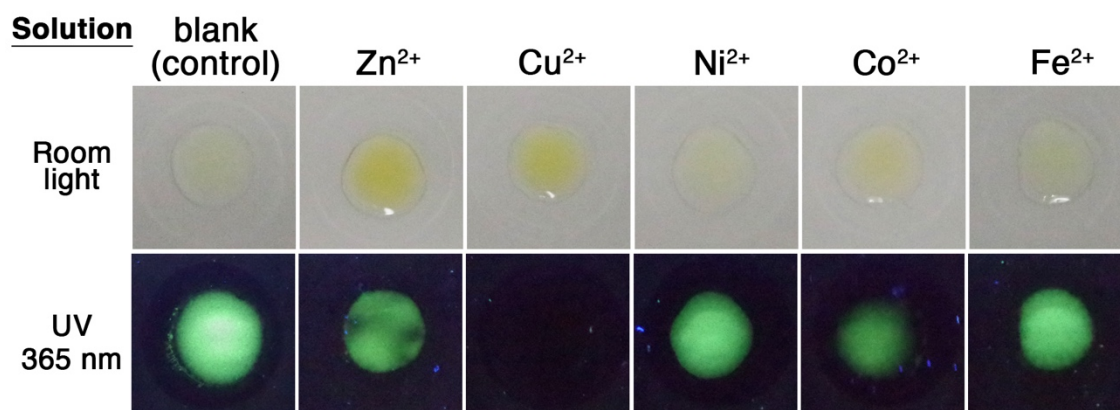


Figure 2-11. Photographs of solution droplets containing 1.0 μg of BF_2 complex **1** in 20 μL of $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (v/v, 1:1) with 3.0 μg of (from left to right) without metal salt (control), $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$, $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$, $\text{Ni}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$, $\text{Co}(\text{OAc})_2 \cdot 4\text{H}_2\text{O}$, and $\text{Fe}(\text{OAc})_2$. (top: under ambient light, bottom: under 365 nm UV light).

Solvent evaporation led to considerable changes in solid color and emission intensity (**Figure 2-12**). A solution drop containing Zn^{2+} ions left an orange solid, and show weak yellow fluorescence in the solid state. Solid spots containing Cu^{2+} , Ni^{2+} , and Co^{2+} salts were non-fluorescent in the solid state. This indicates complexation induced fluorescence quenching of **1**. Whereas Fe^{2+} salt did not affect solid-state emission of **1**. Although the detection limit is rather high (approximately 10 μM), these results clearly show the potential of isopyrazole-based chromophores for metal ion detection. These results show that BF_2 complex **1** is effective for detection of metal ions under fluorescence irradiation conditions. On the other hand, detection limit for metal ions is estimated to be around 10 μM and a few μg scale, which is not particularly good compared to general metal ion sensor materials.

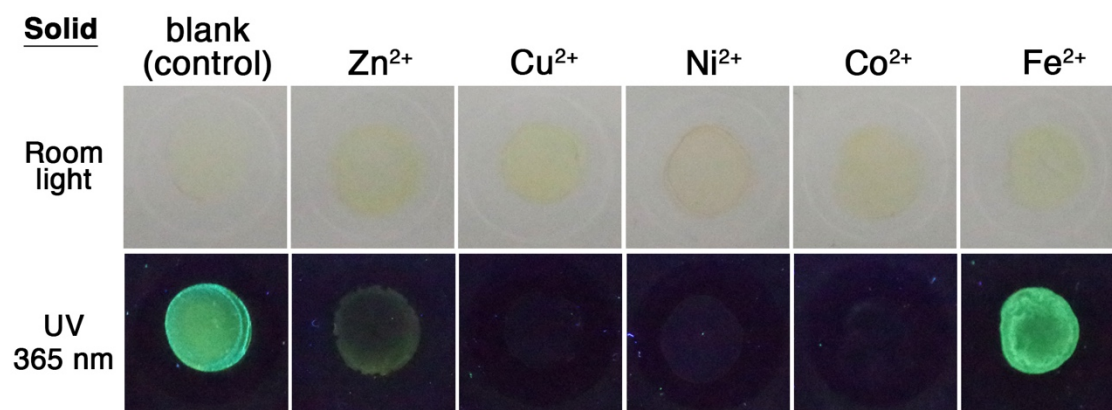


Figure 2-12. Photographs after evaporation of the solvent. (top: under ambient light, bottom: under 365 nm UV light).

2.6. Image-Based Machine Learning Concept for Performance Improvement

I synthesized and developed BF₂ complex **1** with the isopyrazole skeleton, which was confirmed to show some degree of metal ion detection performance, but it is inadequate compared to the other excellent metal ion sensor molecules that have been reported so far.¹⁰⁻¹³ In order to further improve the performance of this molecule, I decided to look at it from a different point of view in molecular design. I reconsidered the results of this study in terms of detection methods.

Although visual observation is considered to be the most rapid and simple method of evaluating the potential for metal ion detection, there are major problems with reproducibility and objectivity due to differences in the criteria used for judging between individuals. For example, in the case of photographs of fluorescence changes or quenching due to the presence of metal ions under UV irradiation as shown in **Figure 2-12**, it is possible for a large number of observers to recognize that the difference in appearance is significant. On the other hand, when focusing on photographs under ambient light, it is possible to recognize slight color changes and unevenness in drying due to the addition of metal ions, but it is difficult to clearly show the detection of metal ions with definitive data. In recent years, image analysis technology has advanced tremendously, and it has become possible to extract a great deal of information from a single image. I suspected that it would be possible to use machine learning techniques to clearly distinguish and quantify the slight differences in photographic data (**Figure 2-13**).

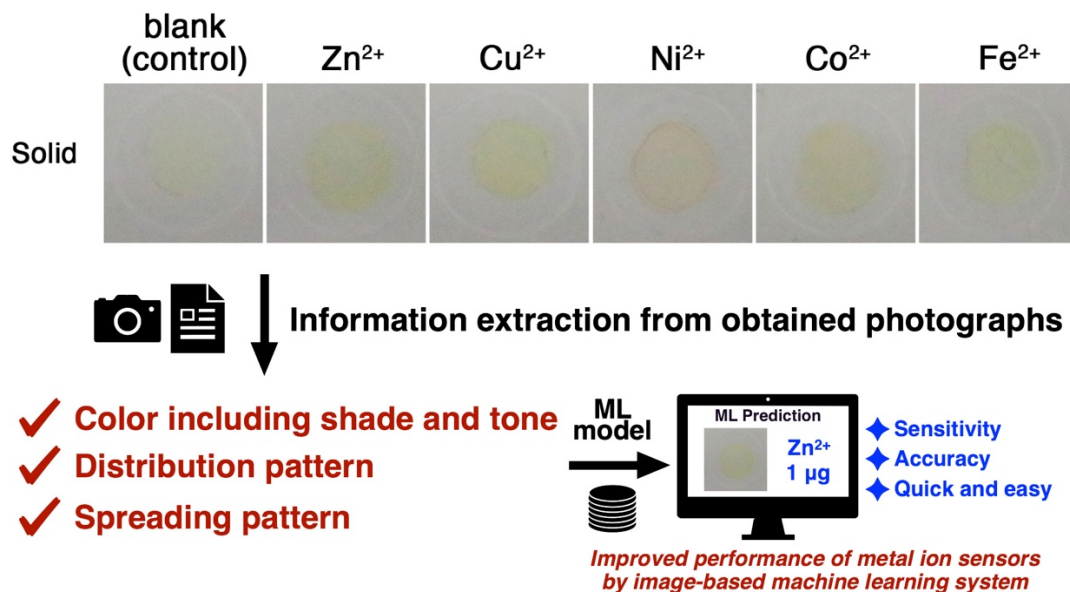


Figure 2-13. Concept of metal ion prediction system using photo and machine learning.

2.7. Conclusion

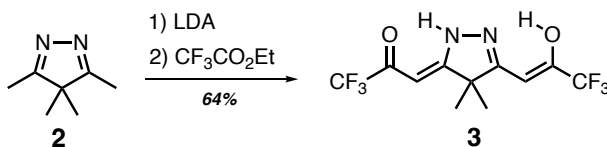
I synthesized BF₂ complex for metal ion sensor, which has strong metal coordination site and fluorescent chromophore for isopyrazole ring via π -conjugation. In organic solvent, yellow-green fluorescence of BF₂ complex changed to yellow in the presence of Zn²⁺ ion, while fluorescence was quenched in the presence of Cu²⁺, Ni²⁺, and Co²⁺ ions. Similar fluorescence change can be observed in solid state even μ g scale of metal ions. Therefore, BF₂ complex have ability of detect and distinguish trace metal ions from solid-state fluorescence. However, detection limit of metal ions was about 1 μ g, because visual changes are difficult to distinguish clearly with naked eye. I thought it may be possible to detect sub- μ g scale using image-based machine learning, because spectral changes can be observed at even smaller equivalent of metal ion. This study was featured in the research-news section of the ICReDD website.^{14,15}

2.8. Experimental Section

General Information

Solvents and reagents were purchased from FUJIFILM WAKO Pure Chemical Industries Ltd., TCI Co., Ltd., and Sigma-Aldrich Co., and used without further purification. All the ^1H and ^{13}C NMR spectra were recorded using a JEOL JMN-ECS400 spectrometer. Chemical shifts are reported in parts per million (ppm) relative to an internal standard tetramethylsilane ($\delta = 0.00$ ppm for ^1H) or residual solvent peak ($\delta = 77.16$ ppm for ^{13}C in CDCl_3). Infrared spectra were measured using a JASCO Co. FT/IR-4700. Elemental analysis was performed using an Exeter Analytical, Inc. CE440. Thin layer chromatography (TLC) was performed on a silica gel sheet, MERCK silica gel 60 F₂₅₄. Preparative scale separations were performed using silica gel gravity column chromatography (Wakosil[®] 60, 64 ~ 210 μm). UV-vis absorption spectra were recorded on a Shimadzu UV-1800 spectrophotometer. Fluorescent spectra were measured on a Hitachi F-7100 spectrometer. Fluorescence lifetimes were recorded on a picosecond fluorescence lifetime measurement system (C11200, Hamamatsu) equipped with picosecond light pulser (C10196), spectrograph (C11119-02), and streak scope (C10627). Quantum yields were measured using a JASCO FP-6300 spectrometer attached with a JASCO ILF-533 integration sphere unit ($\phi = 100$ mm).

Synthesis of Tetraketone Derivative 3

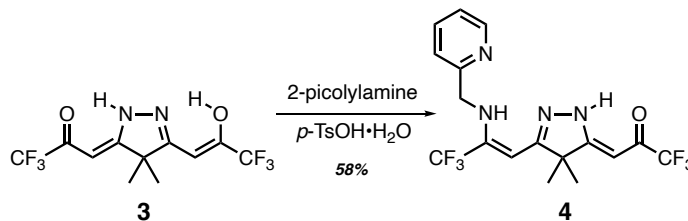


Compound **2** (3,4,4,5-tetramethyl-4*H*-pyrazole) was synthesized by reported procedure.⁸ To a 100 mL round-bottom flask, compound **2** (1.00 g, 8.05 mmol) and anhydrous THF (15 mL) was added under nitrogen atmosphere. The solution was cooled to -78 °C, then THF solution of LDA (2.0 M, 12.1 mL) was added via syringe. The solution was stirred for 10 min at -78 °C. Then CH₃CO₂Et (2.20 mL, 18.5 mmol) was added, and the solution was gradually warmed to room temperature with stirring. After 2 h, reaction mixture was neutralized with 1.0 M hydrochloric acid (35 mL) and transferred to a separating funnel. The water phase was separated and extracted with Et₂O (3 × 50 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (diameter: 6.0 cm, height: 30.0 cm, eluent: gradient CH₂Cl₂/AcOEt = 4:1 to CH₂Cl₂/AcOEt/MeOH = 16:4:1) to give compound **3** (1.63 g, 5.15 mmol) as a yellow solid in 64% yield.

¹H NMR (400 MHz, CDCl₃, 298 K): δ 11.27 (br s, 2H, enol-OH or enamine-NH), 5.67 (s, 2H, vinyl), 1.39 (s, 6H, methyl); ¹³C{¹H} NMR (100 MHz, CDCl₃, 298 K): δ 168.9 (C), 165.4 (q, ²J_{C-F} = 34.6 Hz, vinyl-C), 118.2 (q, ¹J_{C-F} = 285.8 Hz, CF₃), 86.0 (CH), 54.2 (C), 23.9 (CH₃); ¹⁹F NMR (377 MHz, CDCl₃, 298 K): δ -76.03 (s, 6F, CF₃);

IR (ATR, neat): 3253, 2361, 2341, 2327, 1659, 1622, 1573, 1248, 1177, 1136, 1110, 1091, 1054 cm⁻¹; m.p. 137-139 °C (decomp.); HRMS(ESI-TOF): *m/z* [M - H]⁻ calcd for C₁₁H₉F₆N₂O₂, 315.0574, found, 315.0576; Elemental analysis (%): Calcd. for C₁₁H₁₀F₆N₂O₂: C, 41.78; H, 3.19; N, 8.86; Found: C, 41.75; H, 3.12; N, 8.74; *R*_f = 0.33 (eluent: CH₂Cl₂/AcOEt/MeOH = 16:4:1); UV/Vis (CH₂Cl₂) λ_{max} (ε) 377 nm (3.0 × 10⁴ L mol⁻¹ cm⁻¹).

Synthesis of Compound 4

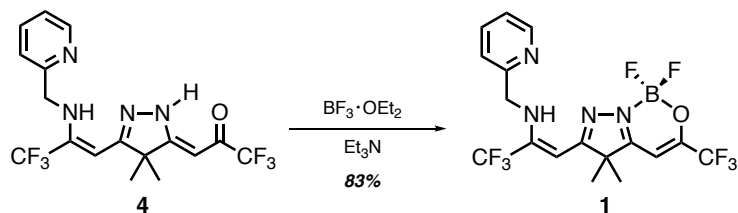


To a 50 mL round-bottom flask equipped with a Dean–Stark apparatus and reflux condenser, compound **3** (500 mg, 1.58 mmol), toluene (5.0 mL), *p*-TsOH·H₂O (180 mg, 0.948 mmol) and 2-picolylamine (240 μL, 2.37 mmol) were added. The reaction mixture was refluxed for 3 h with azeotropic removal of water. After cooling to room temperature, a saturated NaHCO₃ aqueous solution (5 mL) was added, and the reaction solution was transferred to a separating funnel. The aqueous layer was separated and extracted with toluene (2 × 5 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and the solvent was evaporated under reduced pressure. After the evaporation, the resulting residue was chromatographed on a silica-gel column (diameter: 4.0 cm, height: 15.0 cm, eluent: CH₂Cl₂/AcOEt = 7:1) to give compound **4** (375 mg, 0.923 mmol) as a yellow solid in 58% yield.

¹H NMR (400 MHz, CDCl₃, 298 K): δ 12.03 (br s, 1H, enol-OH or enamine-NH), 8.75 (br s, 1H, enamine-NH), 8.65 (dd, *J* = 5.3, 1.9 Hz, 1H, pyridyl proton at C6), 7.71 (ddd, *J* = 7.7, 7.7, 1.8 Hz, 1H, pyridyl proton at C4), 7.29–7.24 (overlapped m, 2H, pyridyl protons at C3 and C5), 5.50 (s, 1H, vinyl), 5.29 (s, 1H, vinyl), 4.72 (d, *J* = 5.6 Hz, 2H, methylene), 1.35 (s, 6H, methyl); ¹³C{¹H} NMR (100 MHz, CDCl₃, 298 K): δ 177.0 (q, ²*J*_{C–F} = 33.4 Hz, vinyl-C), 170.9 (C), 168.0 (C), 156.2 (C), 149.7 (CH), 141.8 (q, ²*J*_{C–F} = 30.6 Hz, vinyl-C), 137.0 (CH), 122.8 (CH), 121.4 (CH), 120.9 (q, ¹*J*_{C–F} = 275.4 Hz, CF₃), 117.8 (q, ¹*J*_{C–F} = 287.5 Hz, CF₃), 83.5 (q, ³*J*_{C–F} = 6.4 Hz, vinyl-CH), 81.0 (CH), 54.5 (C), 49.4 (q, ⁴*J*_{C–F} = 2.9 Hz, CH₂), 24.5 (CH₃); ¹⁹F NMR (377 MHz, CDCl₃, 298 K): δ -66.59 (s, 3F, CF₃), -77.50 (s, 3F, CF₃);

IR (ATR, neat): 3249, 3200, 2977, 2934, 2904, 2874, 2865, 1629, 1594, 1574, 1559, 1509, 1482, 1460, 1427, 1394, 1364, 1242, 1177, 1150, 1129, 1099, 1086, 1035 cm⁻¹; m.p. 144–145 °C; HRMS(ESI-TOF): *m/z* [M + Na]⁺ calcd for C₁₇H₁₆F₆N₄ONa, 429.1121, found, 429.1115; Elemental analysis (%): Calcd. for C₁₇H₁₆F₆N₄O: C, 50.25; H, 3.97; N, 13.79; Found: C, 50.30; H, 3.92; N, 13.64; *R*_f = 0.33 (eluent: CH₂Cl₂/AcOEt = 7:1); UV/Vis (CH₂Cl₂) λ_{max} (ε) 406 nm (3.1 × 10⁴ L mol⁻¹ cm⁻¹)

Synthesis of BF₂ Complex 1



To a 10 mL round-bottom flask equipped with a reflux condenser and an N₂ gas inlet/outlet, compound **4** (200 mg, 0.492 mmol) was added. After the flask was purged with N₂ gas, CH₂Cl₂ (2.0 mL), Et₃N (68.2 μ L, 0.492 mmol), and BF₃·OEt₂ (243 μ L, 1.97 mmol) were added at 0 °C. The reaction solution was warmed to room temperature and stirred for 1 h under N₂ atmosphere. After stirring for 1 h, the reaction solution was quenched with water (5 mL). The reaction solution was transferred to a separating funnel and the organic phase was collected. The water phase was extracted with dichloromethane (2 $\times$ 5 mL), and the combined organic phase was dried over anhydrous Na₂SO₄. The solvent was evaporated using a rotary evaporator. The resulting residue was purified by column chromatography on a silica gel (diameter: 3.0 cm, height: 20.0 cm, eluent: CH₂Cl₂/AcOEt = 15:1) to give compound **1** (185 mg, 0.407 mmol) as a yellow solid in 83% yield.

¹H NMR (400 MHz, CDCl₃, 298 K): δ 10.28 (br s, 1H, enamine-NH), 8.79 (d, J = 4.4 Hz, 1H, pyridyl proton at C6), 7.71 (ddd, J = 7.7, 7.7, 1.8 Hz, 1H, pyridyl proton at C4), 7.30-7.24 (overlapped m, 2H, pyridyl protons at C3 and C5), 6.06 (s, 1H, vinyl), 5.35 (s, 1H, vinyl), 4.83 (d, J = 5.2 Hz, 2H, methylene), 1.43 (s, 6H, methyl); ¹³C{¹H} NMR (100 MHz, CDCl₃, 298 K): δ 177.6 (C), 171.8 (C), 158.6 (q, ² $J_{\text{C-F}}$ = 38.3 Hz, vinyl-C), 154.2 (CH), 149.7 (CH), 145.4 (q, ² $J_{\text{C-F}}$ = 31.6 Hz, vinyl-C), 137.0 (CH), 122.9 (CH), 120.9 (CH), 120.5 (q, ¹ $J_{\text{C-F}}$ = 278.1 Hz, CF₃), 118.5 (q, ¹ $J_{\text{C-F}}$ = 277.5 Hz, CF₃), 87.8 (q, ³ $J_{\text{C-F}}$ = 2.9 Hz, vinyl-CH), 81.7 (q, ³ $J_{\text{C-F}}$ = 6.1 Hz, vinyl-CH), 57.4 (C), 49.0 (q, ⁴ $J_{\text{C-F}}$ = 2.8 Hz, CH₂), 21.9 (CH₃); ¹¹B{¹H} NMR (128 MHz, CDCl₃, 298 K): δ 0.69 (s, BF₂); ¹⁹F NMR (377 MHz, CDCl₃, 298 K): δ -67.65 (s, 3F, CF₃), -74.63 (s, 3F, CF₃), -135.20 to -135.50 (overlapped 2 $\times$ d, ² $J_{\text{F-F}}$ = 12.4 Hz (¹¹BF₂), 2F, ¹⁰BF₂ and ¹¹BF₂); IR (ATR, neat): 3223, 3125, 2994, 2922, 1627, 1598, 1574, 1541, 1493, 1463, 1442, 1425, 1401, 1378, 1357, 1265, 1245, 1211, 1184, 1170, 1146, 1124, 1110, 1064 cm⁻¹; m.p. 158-159 °C; HRMS(ESI-TOF): m/z [M + Na]⁺ calcd for C₁₇H₁₅BF₈N₄ONa, 477.1103, found, 477.1100; Elemental analysis (%): Calcd. for C₁₇H₁₅BF₈N₄O: C, 44.96; H, 3.33; N, 12.34; Found: C, 44.96; H, 3.23; N 12.17; R_f = 0.45 (eluent: CH₂Cl₂/AcOEt = 15:1); UV/Vis (CH₂Cl₂) λ_{max} (ϵ) 418 nm (2.2 $\times$ 10⁴ L mol⁻¹ cm⁻¹)

Single Crystal X-ray Diffraction Analysis

Crystallographic data for compound **3**

Single crystals of compound **3** were grown by vapor diffusion of hexane into CH₂Cl₂ solution of compound **3**.

(C₁₁H₁₀F₆N₂O₂), $M = 316.21$, crystal size: $0.80 \times 0.18 \times 0.17$ mm³, Monoclinic, space group $P2_1/c$, $a = 5.7598(2)$ Å, $b = 14.2374(6)$ Å, $c = 16.5805(7)$ Å, $\beta = 95.842(4)^\circ$, $V = 1352.61(9)$ Å³, $Z = 4$, $T = 123(2)$ K, $\mu = 0.161$ mm⁻¹, $D_{\text{calc}} = 1.553$ g/cm³, $1.890^\circ \leq \theta \leq 26.497^\circ$, 2530 unique reflections out of 2803 with $I > 2\sigma(I)$, GOF = 1.028, $R_1 = 0.0551$ and $wR_2 = 0.1590$ for all data.

CCDC deposit number: 2071576.

Crystallographic data for compound **4**

Single crystals of compound **4** were grown by vapor diffusion of hexane into a diethyl ether solution of compound **4**.

(C₁₇H₁₆F₆N₄O), $M = 406.34$, crystal size: $0.10 \times 0.06 \times 0.05$ mm³, Monoclinic, space group $P2_1$, $a = 8.53283(5)$ Å, $b = 18.10454(9)$ Å, $c = 12.19185(7)$ Å, $\beta = 108.7638(6)^\circ$, $V = 1783.333(17)$ Å³, $Z = 4$, $T = 123(2)$ K, $\mu = 1.239$ mm⁻¹, $D_{\text{calc}} = 1.513$ g/cm³, $3.829^\circ \leq \theta \leq 67.498^\circ$, 6240 unique reflections out of 6425 with $I > 2\sigma(I)$, GOF = 1.029, $R_1 = 0.0274$ and $wR_2 = 0.0665$ for all data.

CCDC deposit number: 2071577.

Crystallographic data for compound **1**

Single crystals of compound **1** were grown by vapor diffusion of hexane into CH₂Cl₂ solution of compound **1**.

(C₁₇H₁₅BF₈N₄O), $M = 454.14$, crystal size: $0.35 \times 0.32 \times 0.21$ mm³, Monoclinic, space group $P2_1/n$, $a = 8.3420(6)$ Å, $b = 18.7176(15)$ Å, $c = 13.0587(10)$ Å, $\beta = 105.889(7)^\circ$, $V = 1961.1(3)$ Å³, $Z = 4$, $T = 123(2)$ K, $\mu = 0.150$ mm⁻¹, $D_{\text{calc}} = 1.538$ g/cm³, $2.176^\circ \leq \theta \leq 25.998^\circ$, 3158 unique reflections out of 3857 with $I > 2\sigma(I)$, GOF = 1.143, $R_1 = 0.0557$ and $wR_2 = 0.1056$ for all data.

CCDC deposit number: 2071578.

Crystal Structures of Compounds by X-ray Diffraction

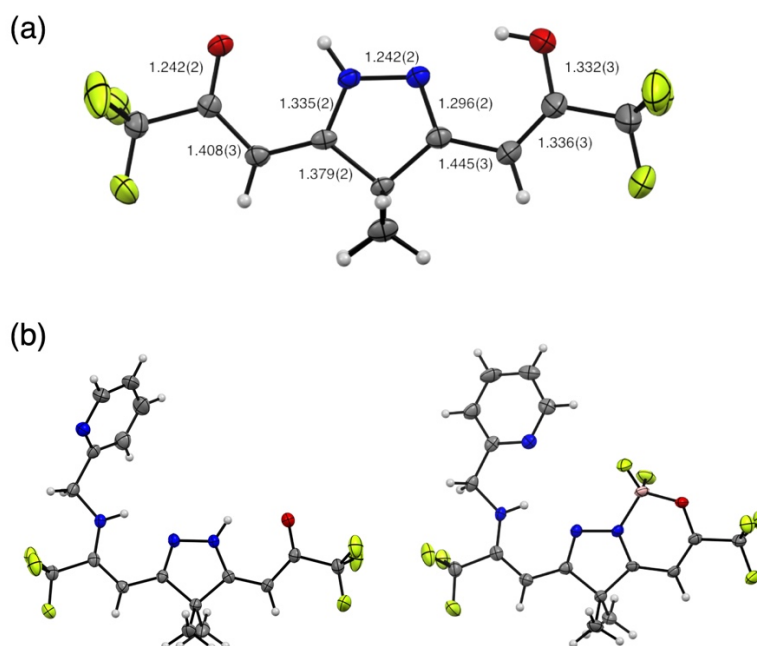


Figure 2-14. (a) Crystal structure and bond lengths (Å) of compound **3**. (b) Crystal structures of compound **4** (left) and **1** (right).

Optical Measurement of Compound **1**

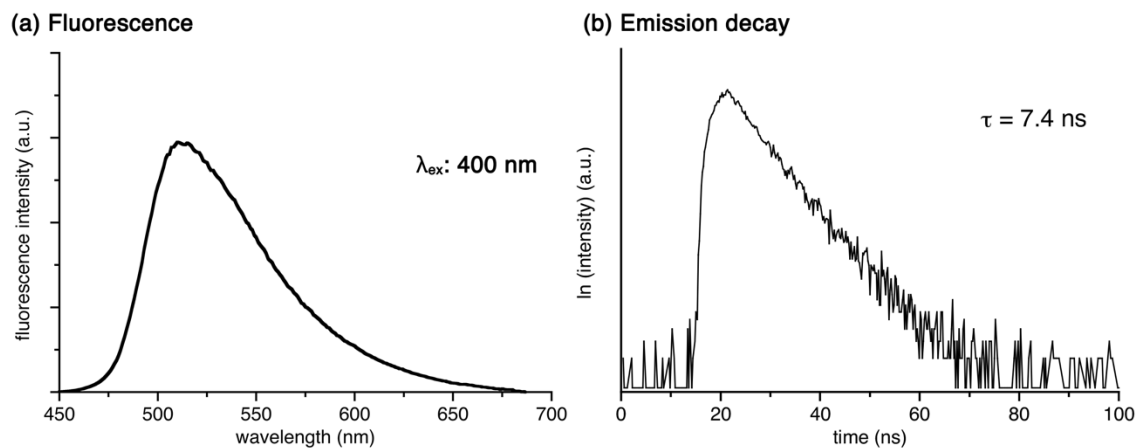


Figure 2-15. (a) Solid-state fluorescence spectrum of compound **1** (excitation wavelength: 400 nm), (b) Emission decay of compound **1** in solid-state.

2.9. References

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<https://www.icredd.hokudai.ac.jp/ja/research/5486> (accessed 2024-12-04)

Chapter 3

Developments of Mixture Ratio Prediction System and Solid-State Reaction Monitoring Using Image-Based Machine Learning

3.1. Introduction

In recent years, image-based machine learning (ML) has been widely applied and has been put to practical use like face recognition, object recognition, and image search.^{1,2} These ML systems can instantly predict information from image data, such as distinguish dogs and cats, by input and analyze large amount of image data in advance. Image-based ML are also applied in the fields of material science and engineering.³⁻⁵ By using ML techniques such as deep learning, automatically extract features from images and construct predictive models. However, ML is not as versatile as people generally say. In general, practical model cannot always be obtained by simply inputting data. It is often difficult for ML to deal with subjects and patterns that are not given or defined as training data.⁶ Accurate prediction results will be difficult to obtain from images taken by different angles, backgrounds, or brightness conditions from training data. Prediction accuracy can significantly reduce if too little or biased data is input (overfitting). Furthermore, when images contain noise or contamination, machine learning can make incorrect or inappropriate predictions, even in cases that most humans can predict them accurately.⁷

It is necessary to establish analytical methods to achieve goal to construct image-based ML system for apply various chemical reactions and improve detection limit for ion sensor. As the simplest prediction target, predict mixing ratio of two-component mixture with different crystal sizes, colors, and shapes each other. Commercially available sugar (sucrose) and dietary salt (NaCl) was selected as a mixture of crystalline compound. Sugar and dietary salt are commonly used as seasonings in daily life, but sometimes mistaken each other by just a quick glance. Since sugar and dietary salt are organic and inorganic compounds, it is difficult to directly quantify them by conventional instrumental analysis. Therefore, sugar/dietary salt mixture was used to prove my hypothesis that image-based ML can distinct chemical compounds.

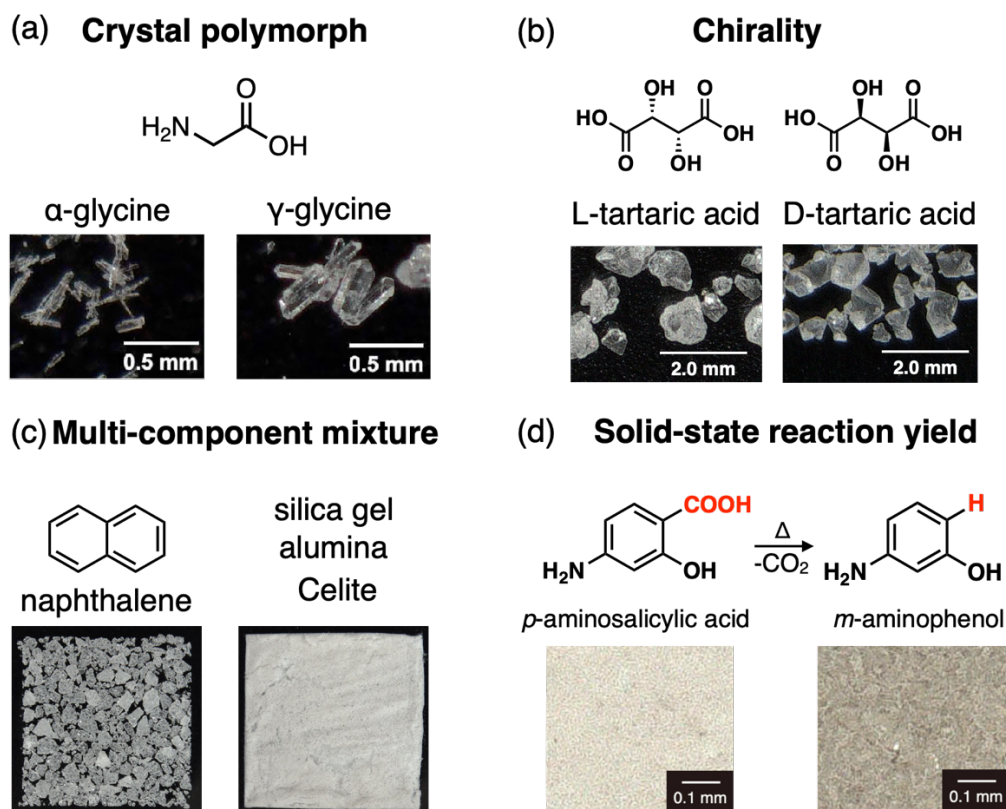


Figure 3-1. Application examples of image-based mixing ratio prediction system.

After investigating optimized conditions, investigated applicability of image-based ML analysis to different chemical solids (**Figure 3-1**). I choose crystal polymorphs, enantiomers, and multi-component organic/inorganic mixture as more difficult subjects to distinguish. α - and γ -glycine was used as an example of a polymorphism whose appearance (shape, color, transparency, etc.) differs depending on the crystal structure. As an example of chiral compound, selected L-/D- tartaric acid which has historical importance in the discovery of chirality. Mixtures containing two or more solid component was predicted for future applications toward complex reaction mixtures. This prediction system was also applied for solid-state decarboxylation of *p*-aminosalicylic acid. By linking post-reaction images to the ^1H NMR yield, try to estimate yield from the sample images. To make ML prediction method more practical, also attempted real-time yield prediction.

3.2. Optimization of Machine Learning Settings

Sugar and dietary salt used in this study are colorless, transparent crystal. Most people would be able to easily distinguish them through a microscope from differences in appearance, such as size, shape, or transparency (**Figure 3-2a**). However, for well-mixed sample (**Figure 3-2b**), it is difficult for human eye to distinguish them, because the grain-size distributions partially overlap (**Figure 3-2c**). Averaged grain lengths of sugar/dietary salt are 0.75 and 0.42 mm and averaged grain weights of 0.17 and 0.14 mg, respectively. It is necessary to find a way to obtain sample images that make it easier to predict the mixing ratio. I explored appropriate conditions such as image data acquisition, machine learning settings, and amount of image data to construct proper models.

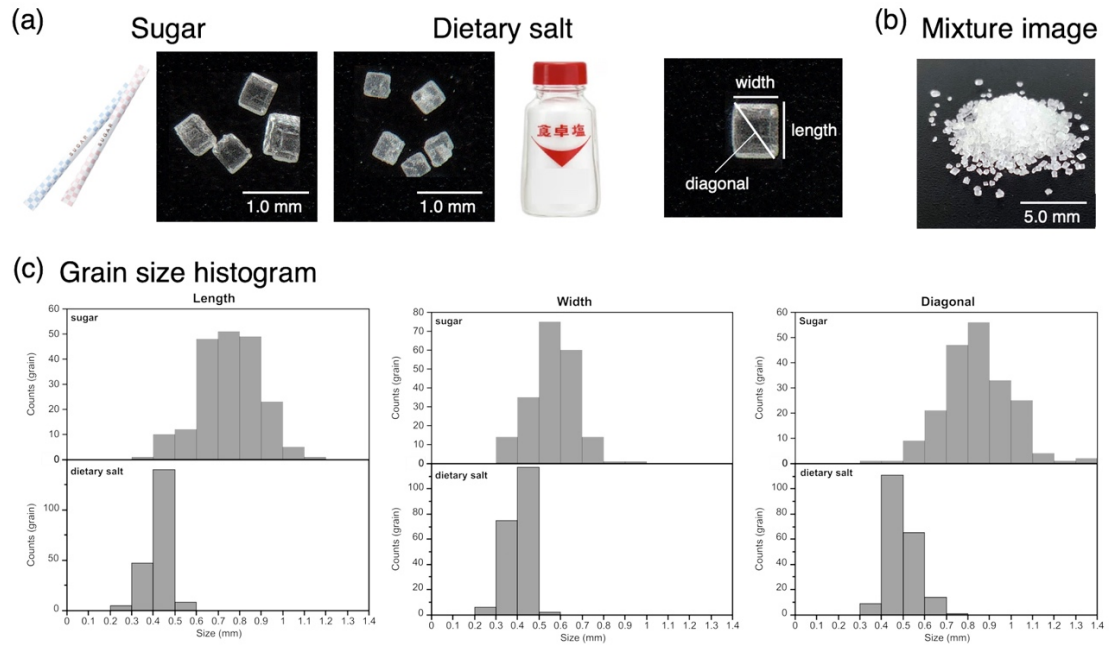


Figure 3-2. (a) Commercially available sugar and dietary salt, and definition of grain size (length, width, and diagonal). (b) Well-mixed sample of sugar and dietary salt used in machine learning experiment. (c) Histograms of size distribution for 200 grains sugar and dietary salt.

For mixing ratio prediction, supervised image-based ML was used in this study. As mentioned in Chapter 1, image itself cannot be used directly as a prediction target. ML model was constructed from relationship between explanatory and objective variables, with numerical features extracted from image data as the explanatory variable and the calculated mixing ratio as the objective variable (**Figure 3-3a**). As the source of these variables, multiple pairs of image data (sugar/dietary salt mixtures) and mixing ratios (sugar : dietary salt weight ratios) are required. Such a pairs of data are called “training dataset” for supervised ML.

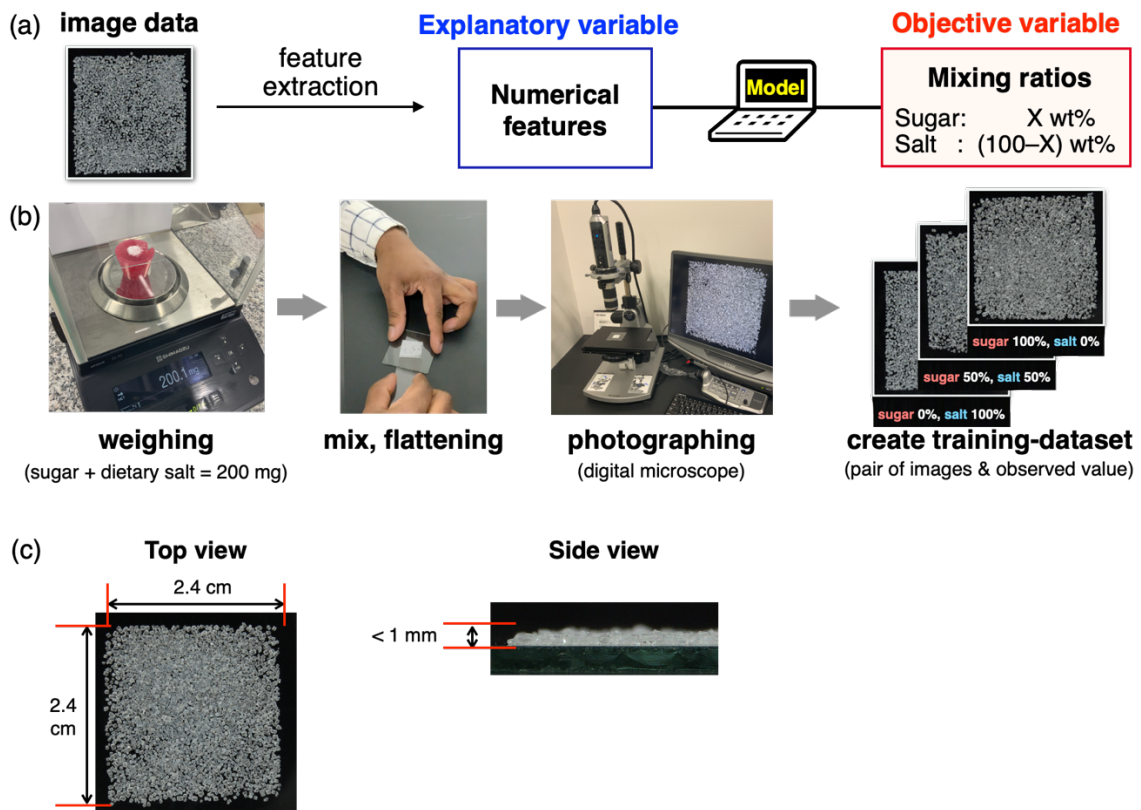


Figure 3-3. (a) Model construction methods for image-based ML. (b) Image acquisition method for ML. (c) Standard to obtain images for sugar/dietary salt mixtures. Sample was flattened to minimize effects of sample overlapping.

100 sample of sugar and dietary salt mixture with different weight ratios was prepared. Sugar/dietary salt (mg/mg) = 0:10, 1:9, 2:8, ..., 9:1, and 10:0 with 9-10 samples for each ratio were weighed and prepared (**Figure 3-3b**). Each well-blended sample (total weight = 200 mg) was spread on a glass plate inside a square area of 2.4×2.4 cm within thickness of less than 1.0 mm so that all solids could be photographed without stacking (**Figure 3-3c**). The glass plate was placed on a black stage to avoid influence of the backgrounds, and image data was obtained with digital microscope camera at resolution of 2048×1536

pixels. Obtained images were trimmed to 1536×1536 pixels squares and paired with actual mixing ratios (weight ratio of sugar : dietary salt) to create training data. A random subset of 10% of the training dataset was used as a validation dataset to choice best model, and the remaining 90% of the training dataset was used for construct model.

Constructions of model generally requires thousands of image data. From the standpoint of practicality and convenience, data augmentation (DA) was performed to construct ML model with a realistically obtainable amount of image data. For DA, image data with the same feature values were amplified by randomly performing rotation, flipping, and section cropping operations from original images. DA was performed using the following methods (**Figure 3-4a**), (i) for each original 1536×1536 pixel image, center cropped to 1200×1200 pixel size to remove the edges of the sample area, (ii) random cropped at different locations to 800×800 pixel size, (iii) random flipping and 90° rotation with a probability of 50%, (iv) contrast normalization by contrast-limited adaptive histogram equalization (CLAHE), (v) resize to 512×512 pixels.

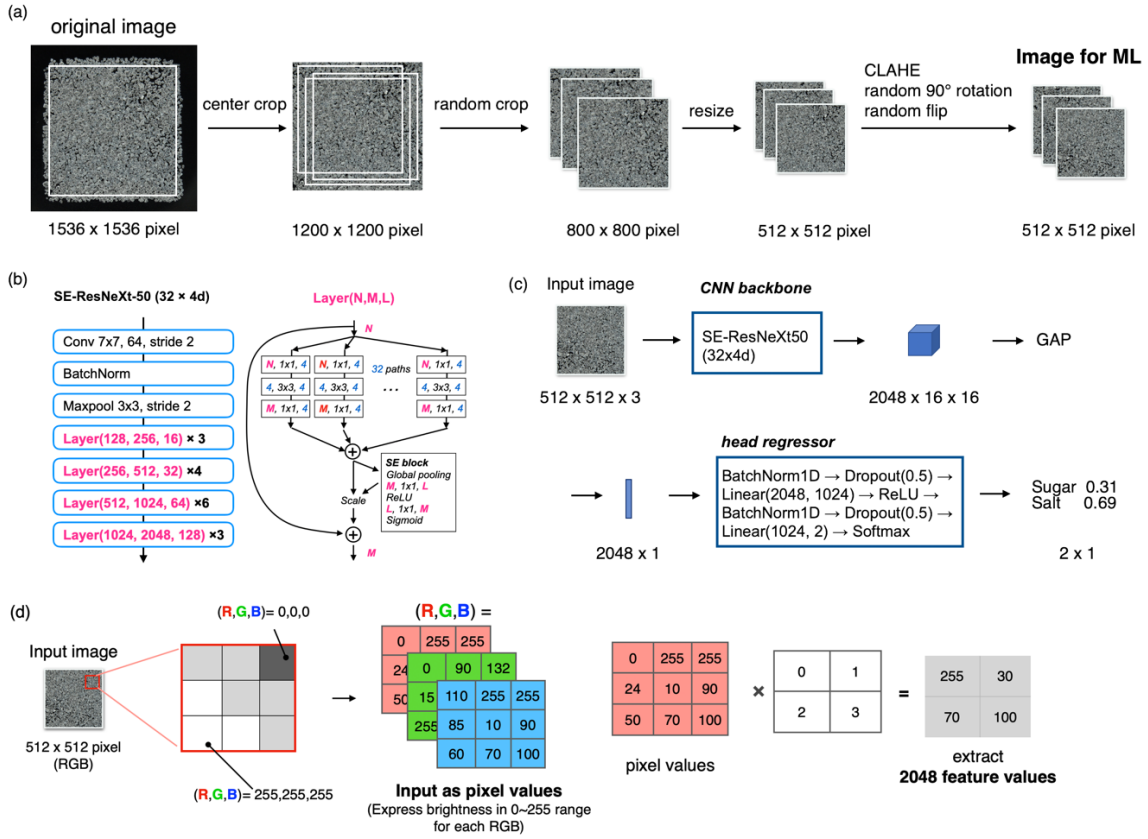


Figure 3-4. (a) Data augmentation (DA) process, (b) Architecture of ML model. (c) Flowchart for construct machine learning model. (d) Feature extraction method from image data.

Features were extracted from subimages obtained by data augmentation, and ML model was constructed using a SE-ResNeXt50 (**Figure 3-4b**), which ResNeXt with SE (Squeeze-and-Excitation) block,⁸⁻¹⁰ as convolutional neural network (CNN) with global average pooling (GAP), head regressor with fully connected layers and Softmax outputs, and it was trained with soft cross-entropy loss (**Figure 3-4c**). For feature extraction, image data is input as pixel values, and feature values are extracted through mathematical processing and used as explanatory variables (**Figure 3-4d**).

For Inference phase, same data augmentation procedure was applied multiple times. The method of applying DA to the Inference phase is called Test-Time Augmentation (TTA). Obtain several 512×512 pixel subimages from a single input image (1536×1536 pixel), and the predicted weight ratios of each subimage were averaged to obtain the final predicted value of the weight ratio of the original input image (**Figure 3-5**). For the sugar/salt two-component example, the ML model output two values as predictions of the weight ratios of sugar and dietary salt in weight%, satisfying $0 \leq \text{sugar (wt\%)} \leq 1$, $0 \leq \text{dietary salt (wt\%)} \leq 1$, and $\text{sugar (wt\%)} + \text{dietary salt (wt\%)} = 1$.

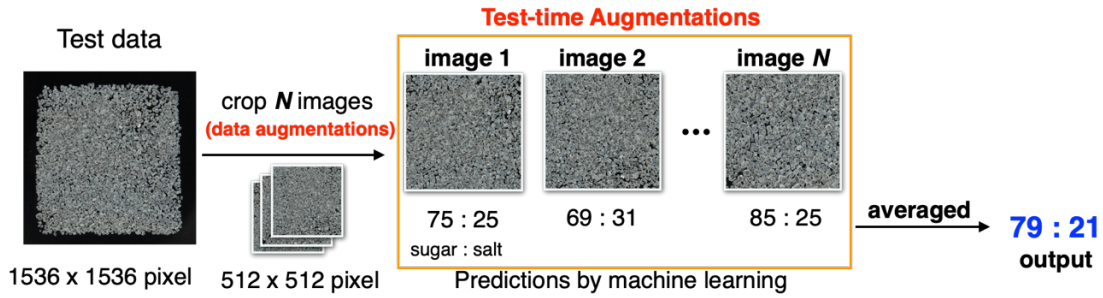


Figure 3-5. Test-time augmentation (TTA) for image-based machine learning.

To evaluate the prediction performance of ML model, another 100 images was prepared and used as “test dataset” to evaluate degree of error between ML predictions and the actual weight ratios. To avoid any unintended data leakage, those test dataset was used as a physically independent data set from the training dataset. In other words, a new sample was taken, weighed, mixed, and spread on a glass plate for each image. So, the test dataset contained the natural batch effect from potential confounders. The prediction accuracy was evaluated by mean absolute error (MAE), root-mean-square error (RMSE), and coefficient of determination (R^2) of observed-predicted plot, with the actual mixing ratios plotted on the horizontal axis and the predictions made by the model plotted on the vertical axis. The closer values of MAE and RMSE are to zero, and the closer R^2 is to 1, the higher prediction accuracies are observed. (in other words, the closer observed-predicted plots are to the diagonal line with positive slope ($y=x$)).

I examined whether sugar/dietary salt mixing ratio predictions could be done under previously mentioned conditions (**Figure 3-6a**). Unless otherwise noted, prediction accuracy was compared by same 100 test dataset created in 1% weight percent increments (sugar/dietary salt (mg/mg) = 0:100, 1:99, 2:98, ..., 99:1, and 100:0). When the machine learning model was directly trained with the input images (with only non-random preprocessing of center cropping to 1200×1200 pixels, resized 512×512 pixels, and CLAHE), the prediction performance was MAE = 10%, RMSE = 11%, and $R^2 = 0.85$ (**Figure 3-6b**).

Although rough relationship between sugar and dietary salt is predictable, prediction accuracy has room for improvement and unstable. This result might be because only 100 training images were input. Adding data augmentation (DA, include random flipping and 90° rotation) improved the prediction accuracy to MAE = 7.1%, RMSE = 9.1%, and $R^2 = 0.90$ (**Figure 3-6c**), without random cropping and TTA. Using data augmentation, practically 12,000 images were given to ML during the training of 120 epochs.

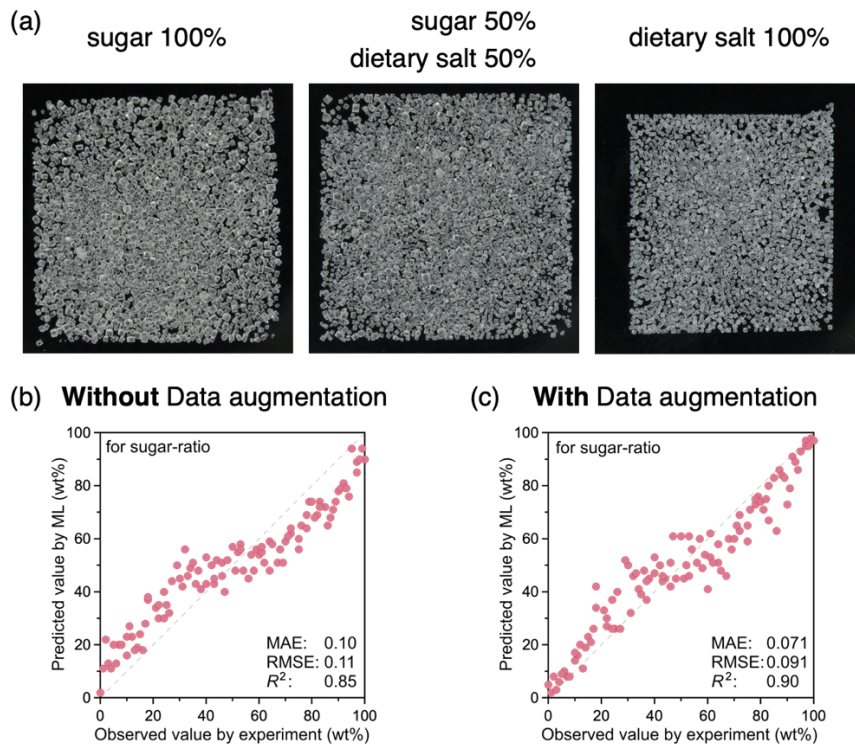


Figure 3-6. Training data :100 images, 10wt% increment, without random cropping and TTA. (a) Digital-microscope images of sugar and dietary salt mixtures with different mixing ratios. (b,c) Observed–predicted plots for 100 test datasets without and with data augmentation (DA).

Given these results, examined effect of adding random cropping and TTA. Times of augmented for each image was changed from 1 to 50 (**Figure 3-7**). As a result, 30-image TTA showed higher performance with MAE = 5.9%, RMSE = 7.7%, and $R^2 = 0.93$ than without TTA. Further increasing TTA did not increase the prediction accuracy. So, I decided to use this condition (DA and 30-image TTA) for further investigations, considering the balance between the efficiency and the significance of the accuracy improvement.

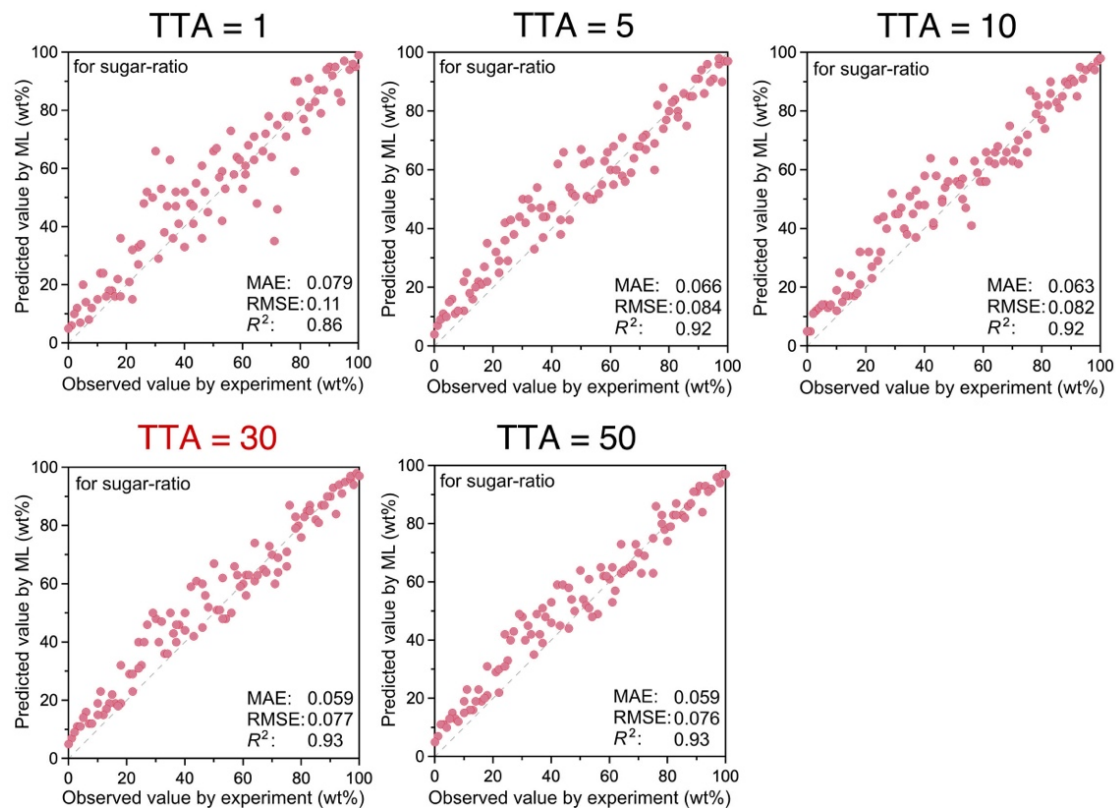


Figure 3-7. Observed–predicted plots for 100 test datasets with random cropping, DA and TTA (training data :100 images, 10wt% increment).

I then optimized image-taken conditions. Accuracy of ML analysis was evaluated using the images with different sample densities (10, 20, and 35 mg/cm²). In each dataset, well-mixed samples were evenly spread over the entire square areas within the dimensions of 2.4 cm × 2.4 cm (**Figure 3-8**). Results of ML shows no significant differences in MAE, RMSE, and R^2 (**Figure 3-9**). However, 10 mg/cm² data showed some outliers in the observed–predicted plot, and the predictions with 20 mg/cm² data were underestimated in the range of higher sugar contents (≥ 60 wt%). Although these sample density conditions are useful for a rough estimation of mixing ratios when the number of authentic samples is limited, I chose the densest setting (35 mg/cm²) as the best condition for image-taking.

The reason for low accuracy may be due to non-uniformity of samples, since the original images were randomly cropped for learning and analysis by DA and TTA processing. In addition, high percentage of background area for input image may cause slight differences in the background color to be learned.

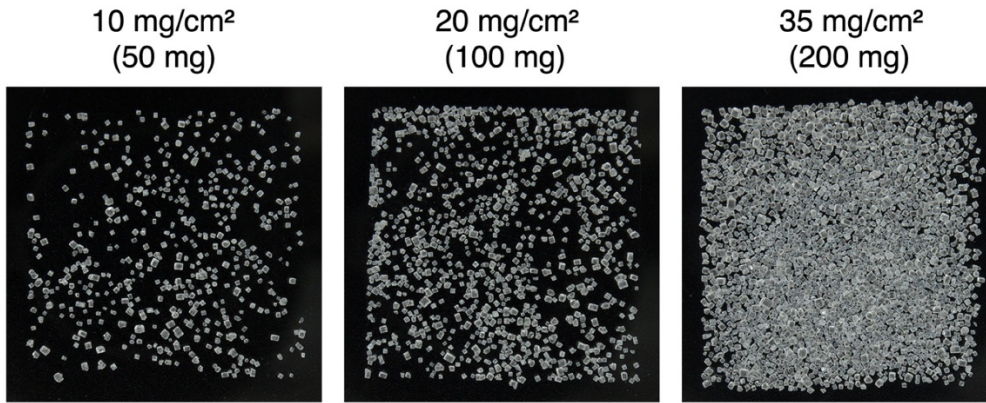


Figure 3-8. Digital-microscope images of sugar and dietary salt mixtures with different sample densities (sugar : dietary salt = 50 : 50).

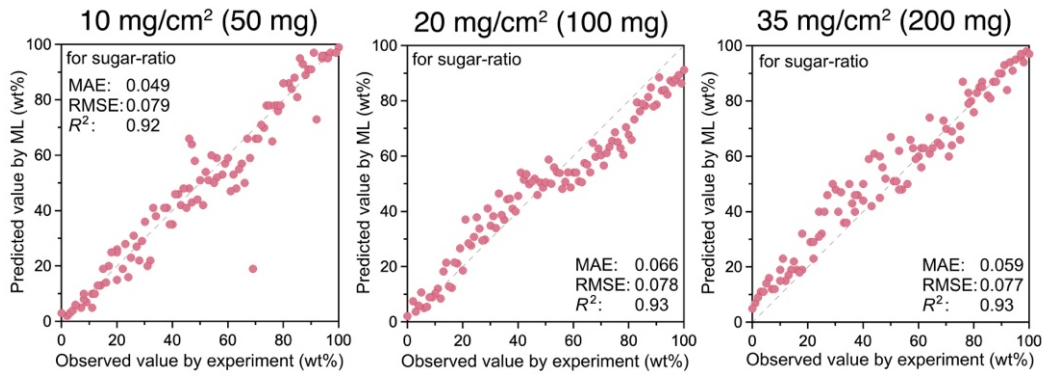


Figure 3-9. Observed–predicted plots for 100 test datasets under different sample densities (training data : 100 images, 10wt% increment). Each test data consisted of 100 independent images taken under the same conditions as each training data.

Furthermore, compared ML model prediction accuracy by training dataset for different increments (1, 10, and 20 wt%, **Figure 3-10**). For the 1 wt% increment, the sugar/dietary salt weight ratios (total: 200 mg, mg/mg) were 0:100, 1:99, 2:98, ..., 99:1, and 100:0. For the 20 wt% increment, sugar/dietary salt weight ratios (total : 200 mg, mg/mg) were 0:100, 20:80, 40:60, 60:40, 80:20, and 100:0 (16~17 samples for each ratio). All the training datasets consisted of 100 images. The DA, TTA, and ML settings were applied under the same conditions. The prediction accuracy was evaluated by the same dataset (100 images, 1 wt% increment). Training dataset with 1 wt% increments show lowest MAE and RMSE value than those of 10 and 20 wt% increments (**Figure 3-11**). Although ML can complement data that have not actually been input for training processes, preparing various mixing ratios for training was found to be effective for model construction.

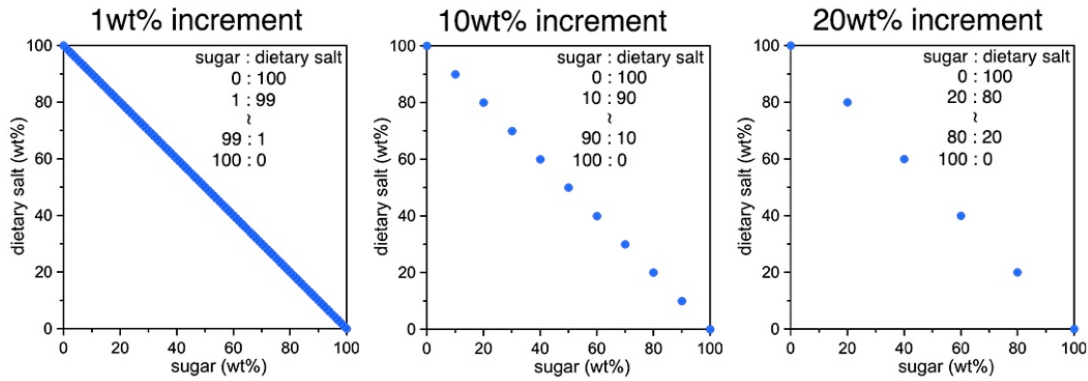


Figure 3-10. Comparison of the sample ratio increments in the 100 training images.

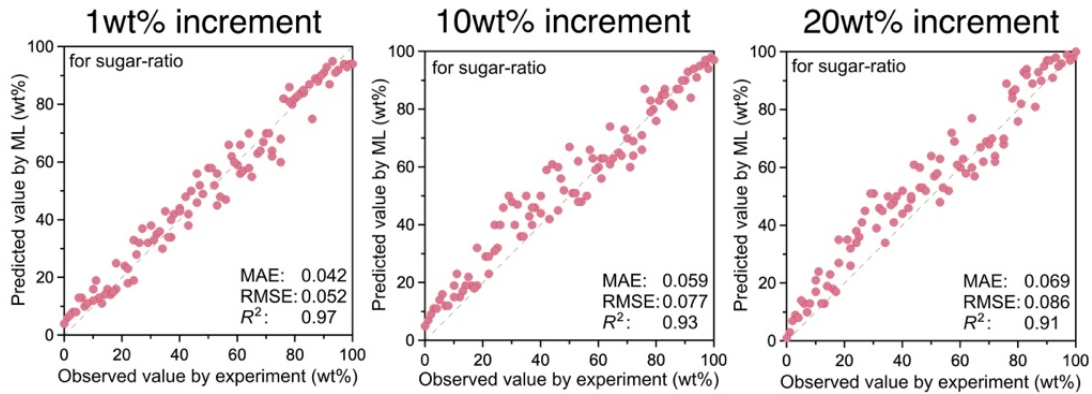


Figure 3-11. Observed–predicted plots for 100 test datasets under different sample increments (training data :100 images).

I investigated the relationship between the number of training images and the prediction accuracy to evaluate the cost-performance ratio, under optimized conditions (30-image TTA, 35 mg/cm², and 1 wt% increment). Prepared training datasets with different numbers of images (10, 25, 50, 100, 200, 300, 400, 500 images) and prediction accuracy was evaluated by the same dataset (100 images, 1 wt% increment). When only 10 images were used as training data, prediction was completely failed, but moderate-level prediction was achieved using just 25 images (**Figure 3-12a**). From 25 to 300 images, prediction accuracy increases monotonically as the number of training images increased (MAE = 3.9%, RMSE = 4.6%, and $R^2 = 0.97$ for 300 images). Even numbers of images were increased to 400 or more, no improvement in prediction accuracy was observed. From the perspective of cost-effectiveness, 300 images of training dataset were determined as the optimal condition for this sugar-dietary salt mixture (**Figure 3-12b**).

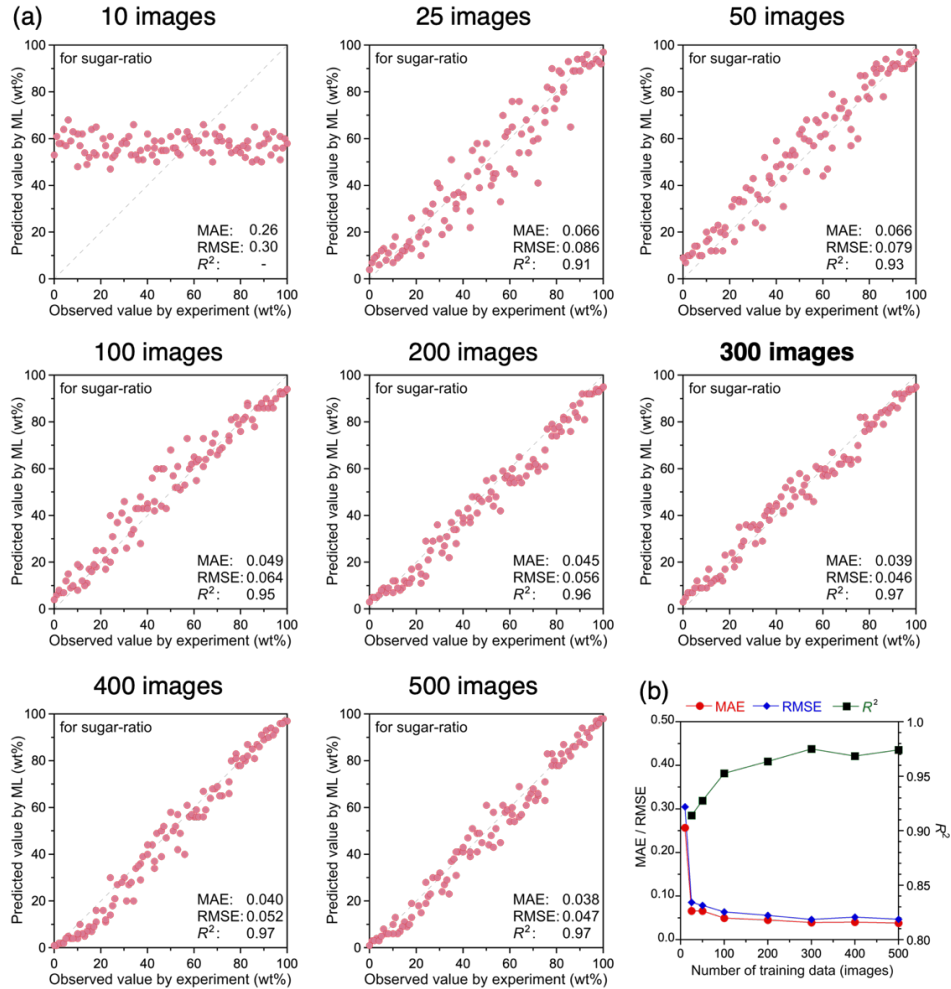


Figure 3-12. (a) Observed–predicted plots for 100 test datasets using different numbers of training images (training data: 1% increment). (b) Relationship between the training image data and the prediction accuracy.

Examples of prediction results under the optimized condition are shown in **Figure 3-13**. It might be difficult for human to instantly see overall images and predict sugar/dietary salt ratio, image-based ML can make quick inferences. The inference time for computing the predicted values for 100 images was 34.79 second with a 30-image TTA on an NVIDIA A100 GPU (0.348 second per image), 7.52 second with one-image TTA (0.075 second per image) on a NVIDIA A100 GPU, and 20.25 second with one-image TTA on an AMD EPYC 7252 3.1 GHz/8-core CPU (0.202 second per image).

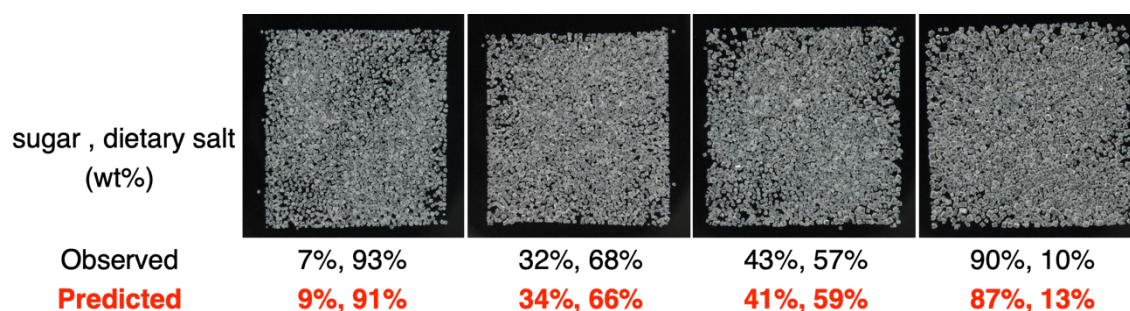


Figure 3-13. Example of mixing ratio prediction under optimized conditions (Training data : 300 images, 1 wt% increment, with DA and TTA).

3.3. Comparison of Machine Learning Prediction with Human-Eye

To confirm the usefulness of the machine learning system, the prediction accuracy of the sugar and dietary salt ratio was compared with human-eye prediction by five participants, consisting of four non-experts (human 1~4, unrelated to this research project) and author (human 5). To make the conditions similar to the optimized prediction system, the five examinees performed mixing ratio predictions on 10 randomly mixed images, referring to the training dataset of 300 images. The results (**Figure 3-14**) indicated that the accuracy of human predictions varied greatly by individuals (MAE = 7.5%–25%, RMSE = 9.3%–34%, $R^2 \leq 0.88$). The prediction accuracy by ML model (MAE = 4.2%, RMSE = 5.6%, $R^2 = 0.96$) was superior to all human-eye prediction with high accuracy and reproducibility. In addition, while it took about 5-10 minutes to make a prediction with human-eye prediction, machine learning successfully predicted the same 10 images in 5-10 seconds. The importance of ML is demonstrated not only by the speed and accuracy of predictions, but also by the fact that machine learning does not "tire" like humans, in other words, can continue to predict huge number of samples with stable accuracy.

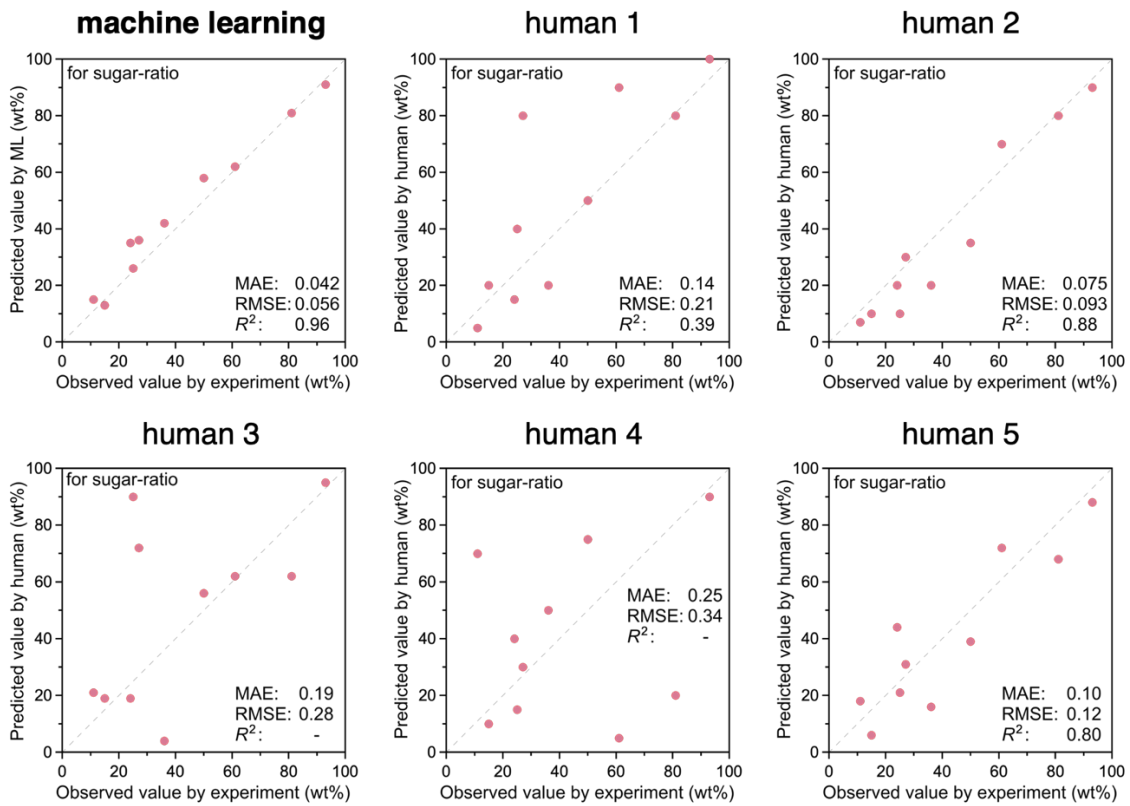


Figure 3-14. Observed–predicted plots for 10 randomly mixed test images by (a) optimized ML prediction system (training data: 300 image) and (b) five examinees.

To confirm the reproducibility and further expand the ease of applicability, developed a stand-alone prediction system that can be used by any external nonexperts for third-party evaluation. Once a suitable machine learning model for a given task is obtained by training, it can be shared with others and separately used for making predictions of any unseen images of the task. Demonstration of the sugar/dietary salt example with instructions for sample preparation and photographing is provided as a freely accessible Google Colaboratory notebook (**Figure 3-15a**). The machine learning program can predict the sugar/dietary salt mixing ratio from a single image (default settings: warm up, 20; up train, 100; TTA, 30; flip, ON; plot text, OFF).

This stand-alone prediction system was tested by a person unrelated to this ML research to demonstrate its usefulness. I informed the participant about the 10 different ratios of the sugar and dietary salt mixtures. The participant collected images of the sugar/dietary salt mixture according to the instructions provided in the stand-alone prediction system on Google Colaboratory. The captured image was centered trimmed (1536×1536 pixels) and converted to the compressed image format (.jpg). Prediction results (MAE = 4.1%, RMSE = 4.9%, $R^2 = 0.95$, **Figure 3-15b**) indicated that anyone can easily perform image-based machine learning predictions by simply uploading the prepared images onto the stand-alone system and clicking according to the instructions.

This stand-alone prediction system is easy to use even for people who don't have detailed knowledge of chemistry, programming, or machine learning. Although it should be carefully operated for input test images to match the training conditions (like crystal size or morphology), this result suggests that image-based ML models can be used as a storage tool for visual information from experiments to be shared among researchers.

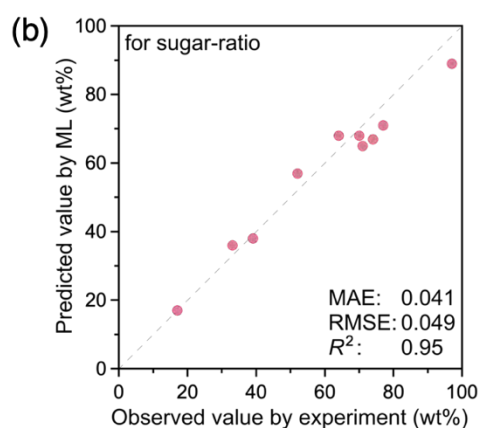
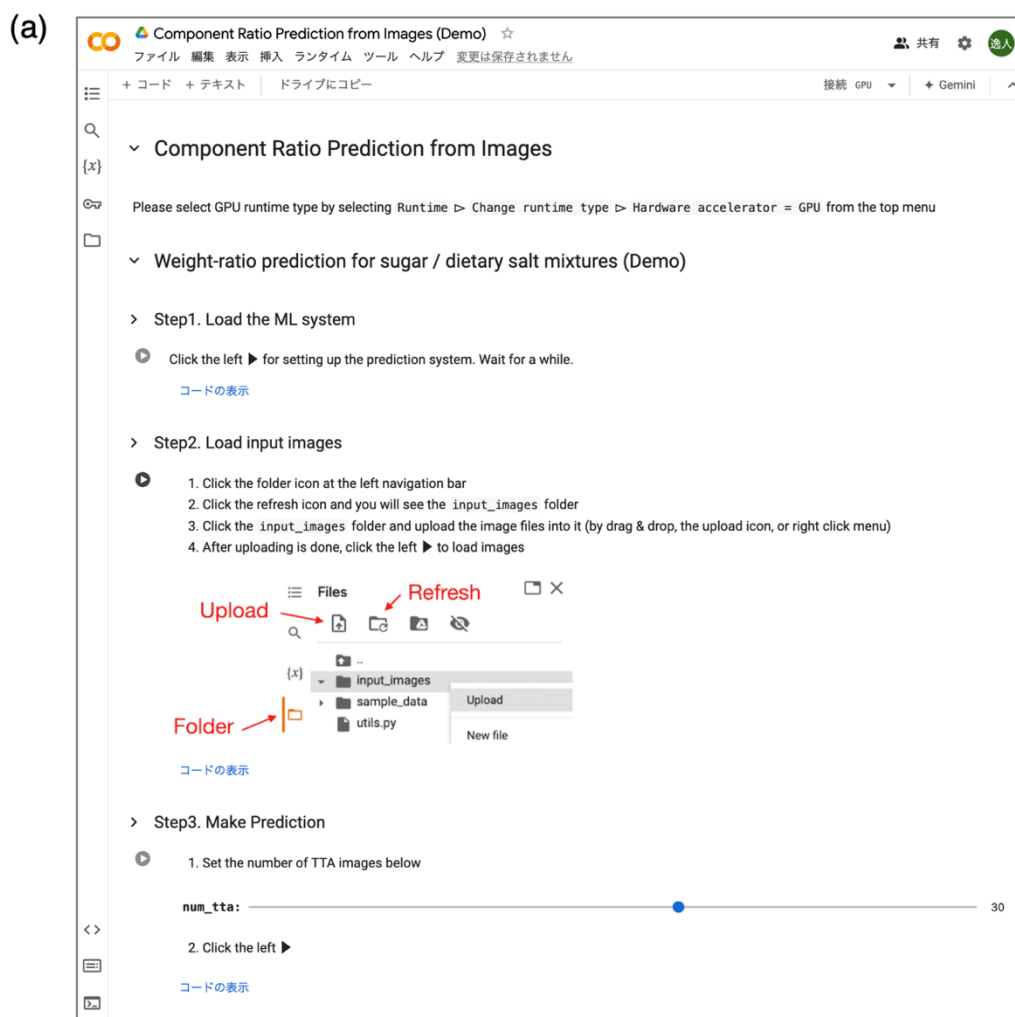


Figure 3-15. (a) ML prediction system on Google Colaboratory. (b) Observed–predicted plot for 10 randomly mixed test images created by third person, from ML prediction system on Google Colaboratory.

3.4. Prediction by Mobile Camera Images

To increase the usefulness of ML, it is desirable to make predictions from images taken with mobile cameras such as smartphones. The mobile camera pictures were taken with an iPod Touch (7th generation, Apple Inc.). The images (**Figure 3-16**) captured by the digital microscope and mobile camera had different image resolutions (pixels and ppi). Images were collected according to the criteria described in Section 3.10. and cropped to create images suitable for prediction.

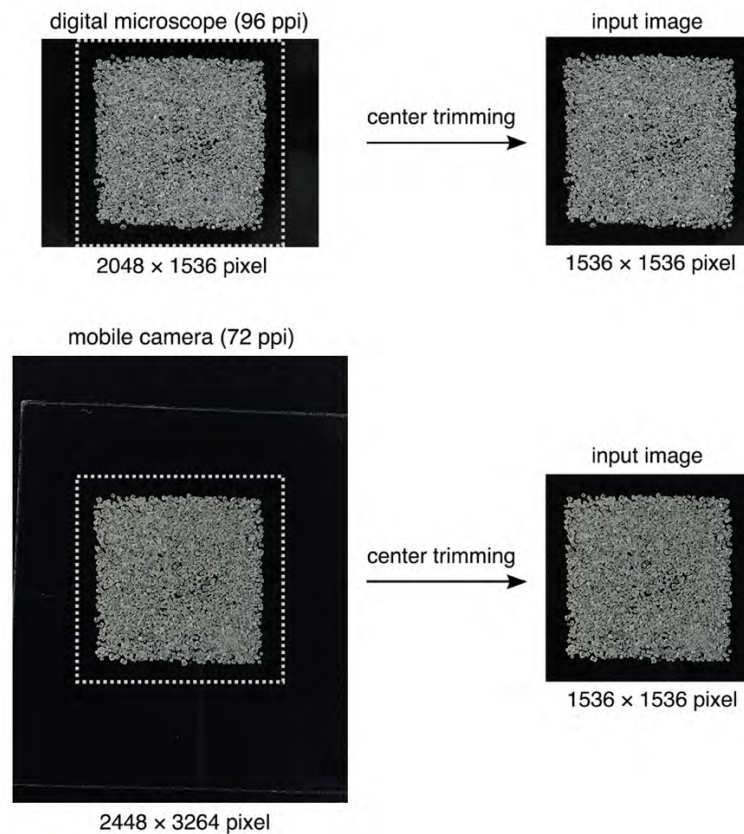


Figure 3-16. Original images captured by a digital microscope and a mobile camera, and the trimmed images for use in the image-based machine learning system.

Under the optimal conditions of the digital microscope, I predicted the sugar/salt mixture ratio of images taken with a mobile camera (**Figure 3-17a**). The model construct with 100 training data showed almost same performance (MAE = 4.9%, RMSE = 6.1%, $R^2 = 0.96$, **Figure 3-17b**) as those obtained with the digital microscope (**Figure 3-12**).

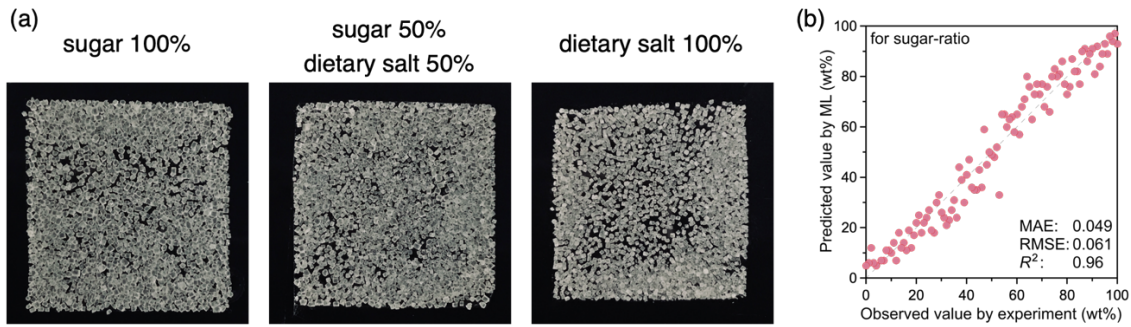


Figure 3-17. (a) Mobile camera images of sugar and dietary salt mixtures with different mixing ratios. (b) Observed–predicted plots for 100 test datasets (training data : 100 images, 1% increment).

To demonstrate the system's ease of use, training data images were taken under less rigorous conditions. Pre-weighed samples in vials were spread on a piece of black cardboard, and the entire sample volume was adjusted to fit within the capture area (**Figure 3-18a**). Although the accuracy of the predictions was slightly lower (MAE = 8.1%, RMSE = 13%, $R^2 = 0.83$, **Figure 3-18b**), rough predictions were shown to be correct.

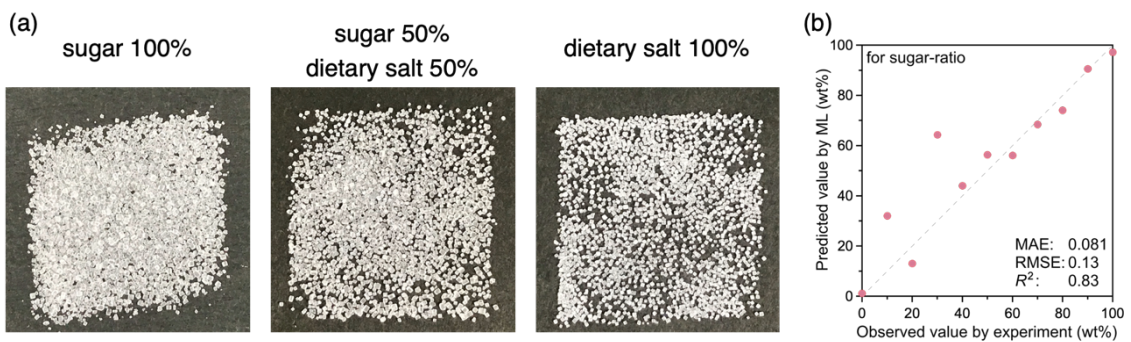


Figure 3-18. (a) Mobile camera images of sugar and dietary salt mixtures taken under less rigorous conditions. (b) Observed–predicted plots for 11 test datasets (training data : 300 images, 10% increment).

Although image-based ML is applicable to analyze images of various solid mixtures, dependence on the type of camera can be a common limitation. Prediction performance could fall substantially from image data taken by different camera. For the six test images taken by the mobile camera, the MAE, RMSE, and R^2 of the machine learning model trained on 300 microscopic images were 19%, 22%, and 0.58, respectively (**Figure 3-19a**). This can be attributed to the subtle differences in the image resolution and brightness between the mobile camera and the digital microscope. When the machine learning model pre-trained with 300 microscope images was fine-tuned by additional training with 24 mobile-camera images for calibration, the MAE, RMSE, and R^2 improved to 6.0%, 6.4%, and 0.97, respectively (**Figure 3-19b**). The ability to use different cameras for image-based machine learnings may allow for the integration of very large training datasets built in different laboratories towards the same target to construct more accurate models.

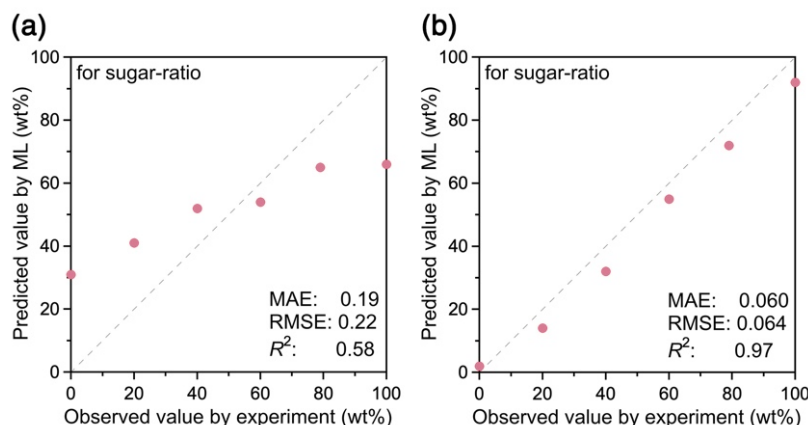


Figure 3-19. Observed–predicted plots of 6 test images taken by mobile camera using different training processing. (a) Trained by 300 digital microscope images. (b) Pre-trained by 300 digital microscope images and trained by 24 mobile camera images. The machine learning model trained by digital microscope images was constructed under the same conditions as in Figure 3-11, and the mobile camera images were acquired under conditions as in Figure 3-14.

3.5. Prediction of Crystal Polymorph for α -/ γ - Glycine

Crystalline polymorphs are compounds with the same chemical composition but have different crystal structures. It is an important concept especially in the field of pharmacology because physical properties (such as rate of dissolution) are different from each other.¹¹ Polymorphs are composed of the same molecules, and polymorphic information is lost by dissolution, limiting qualitative and quantitative analysis methods. Glycine, a simple amino acid, exists two stable crystal polymorphisms at ambient temperature and pressure. When glycine is crystallized from pure water, α -glycine exclusively obtain in the monoclinic $P2_1/n$ space group. Conversely, crystallization from hydrochloric acid gives γ -glycine in the trigonal $P3_1$ space group (**Figure 3-20**).¹² These two polymorphs have different appearance, such as solid shape derived from the crystal structure.^{13,14} Therefore, image-based ML may be able to distinguish between these polymorphisms.

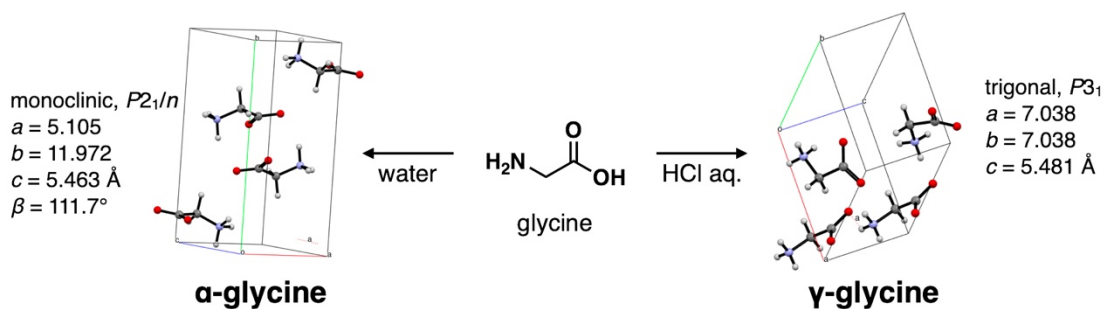


Figure 3-20. Crystal structure of α - and γ -glycine obtained from aqueous solution under ambient temperature and pressure.

To ensure the purity of crystal polymorphism, recrystallization of glycine was conducted by a reported procedure.¹² FT-IR and powder X-ray diffraction (PXRD) analysis were performed to confirm obtained crystal polymorphs. The peak patterns of the spectra were different from each polymorph and in agreement with literature, so pure α - and γ -glycine were successfully obtained (see **Figure 3-39** and **Figure 3-40** in Section 3.10.). The appearance of obtained crystals are shown in **Figure 3-21a**. The two crystals look different; α -glycine is rod-shaped, and γ -glycine is prismatic.

Training dataset was created based on method for sugar and dietary salt. Using authentic solids of α - and γ -glycine, 100 images of mixtures with different weight ratios (total 200 mg, created in 1% increments) were taken as the training data set for machine learning (**Figure 3-21b**).

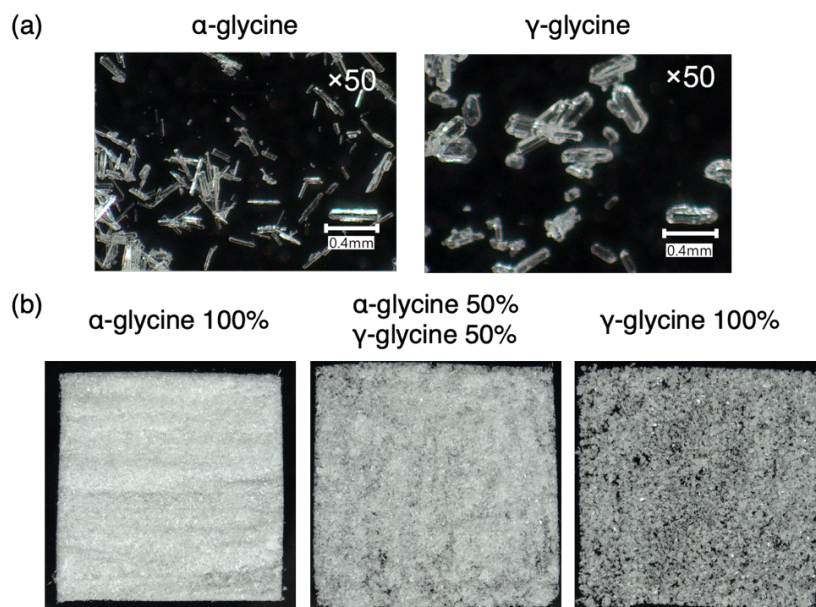


Figure 3-21. α - and γ -glycine crystallized from aqueous solution. (a) Scale and shape of each crystal. (b) Digital-microscope images used for machine learning (200 mg).

The ML pipeline for the initial pilot case was applied to this data set, and its accuracy was evaluated using another physically independent set of 100 images as the test dataset. **Figure 3-22a** shows a graph plotting the predicted values on the vertical axis and the weighed values on the horizontal axis. When 100 sample images were used as training data, high correlation was confirmed between the predicted values and the weighed values (MAE = 5.7%, RMSE = 7.4%, $R^2 = 0.93$). Increasing the amount of training data to 300 images (sugar and salt optimum conditions) further reduced the prediction error (MAE = 4.0%, RMSE = 5.6%, $R^2 = 0.96$). Example of prediction result was shown in **Figure 3-22b**. A reported method using infrared spectroscopy for the evaluation of α - and γ -glycine has an uncertainty of 2%.¹⁵ ML predictions showed that polymorph ratios could be predicted using images with an accuracy comparable to infrared spectroscopy.

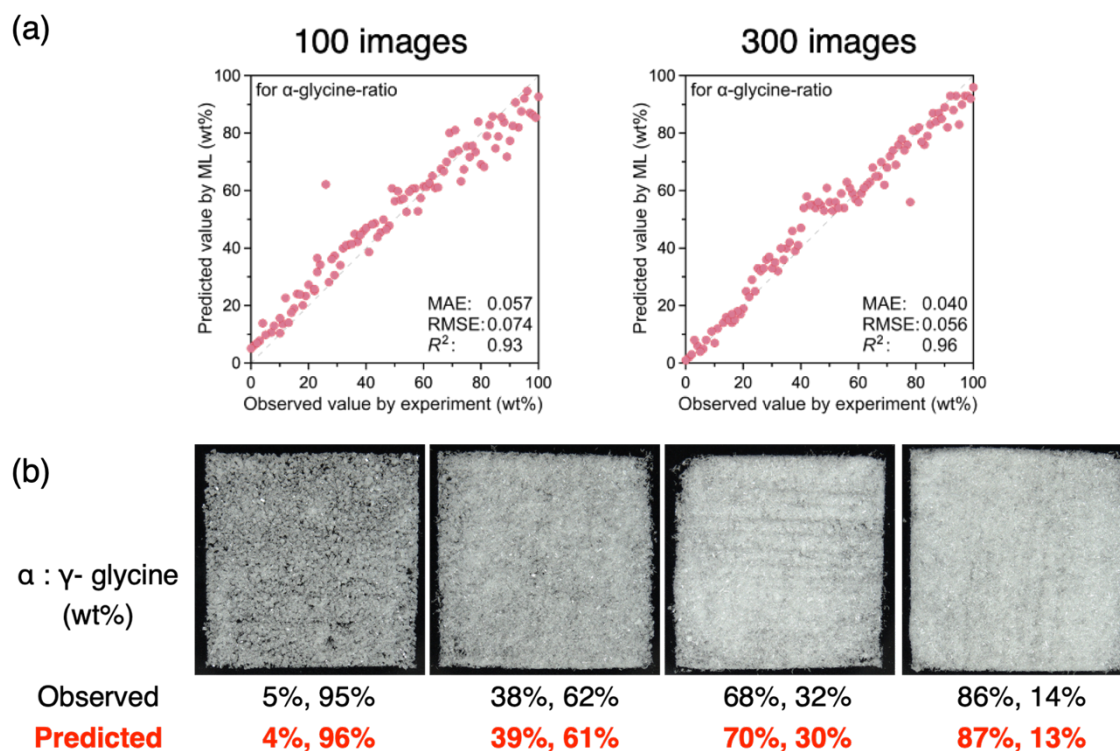


Figure 3-22. (a) Observed–predicted plots for 100 test datasets using different numbers of training images (training data: 1% increment). (b) Example of crystal polymorph ratio prediction under optimized conditions (training data : 300 images).

3.6. Prediction of Enantiomeric Ratio for L-/D- Tartaric Acid

Enantiomers, two stereoisomers in a mirror-image relationship, one of the most difficult combinations to distinguish in molecular science. Enantiomer is also important concept for historically. In the 19th century, Louis Pasteur succeeded in the optical resolution of tartrates by visual inspection, leading to the discovery of molecular chirality.¹⁶ The purity of an enantiomer is described by the enantiomeric excess or enantiomeric ratio (*er*),^{17,18} an important index that is frequently used to evaluate the outputs in asymmetric synthesis.

To predict *er* (in other words, ratio of each enantiomer) by image-based ML, prepared 300 different mixtures of an enantiomer pair: L- and D- tartaric acids. Unlike sugar and dietary salts, the crystalline solid of L- tartaric acid shows almost the same color and size distribution as its enantiomer, D-tartaric acid (**Figure 3-23a**). Image-taken method was also carried out under the same conditions as for sugar and dietary salt (**Figure 3-23b**).

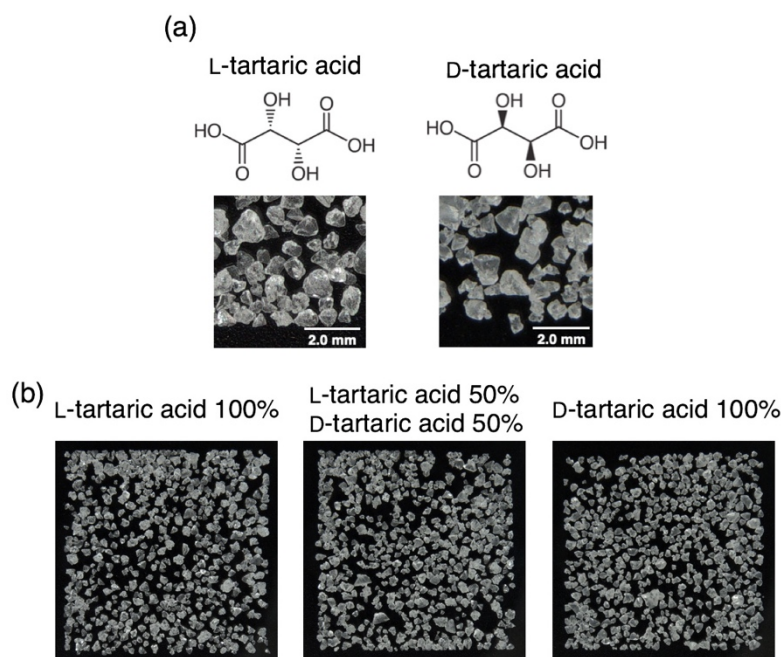


Figure 3-23. L- and D- tartaric acids. (a) Scale of each crystal. (b) Digital-microscope images used for ML (total weight : 200 mg).

To distinguish between the enantiomers, ML was performed in appropriate situations, excluding random flipping during data augmentation. The observed-predicted plot (**Figure 3-24a**) for another independent test data set of 100 images showed that from the images of crystalline mixtures, image-based machine learning can predict the *er* with MAE, RMSE, and R^2 of 6.4%, 7.9%, and 0.93, respectively. (**Figure 3-24b**) Although the appearances of enantiomeric crystals are too confusing to quantitatively distinguish with the human eye, image-based ML can rapidly and quantitatively determine the *er*.

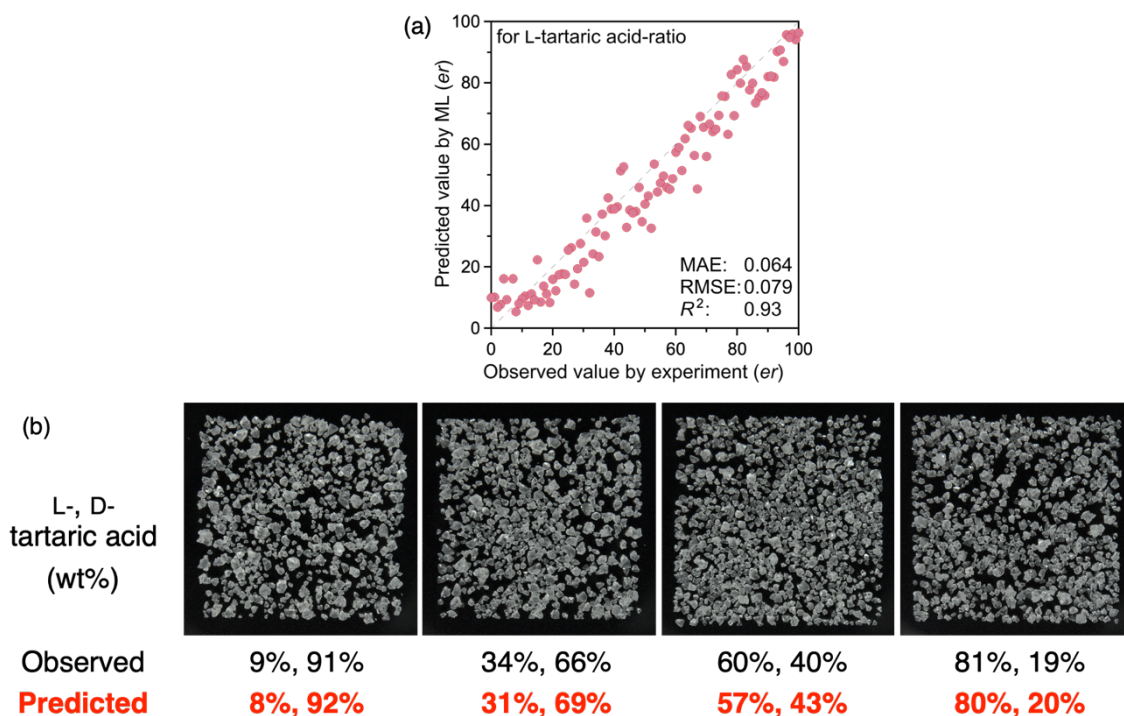


Figure 3-24. (a) Observed-predicted plots for 100 test datasets. (training data: 1% increment, 300 images) (b) Example of enantiomeric ratio prediction under optimized conditions.

3.7. Prediction of Mixing Ratio for Multicomponent Mixtures

In general, crude product of an organic chemical reaction is a mixture of the target compound and small number of impurities that are difficult to identify. As the first step in applying this machine learning technique to quantitative analysis of chemical reactions, I attempted to predict the mixing ratio between the target product (organic compound crystal) and several impurities (inorganic compounds powders) mixed in random proportions.

As a model case, I prepared 300 samples of naphthalene, silica gel, alumina, and Celite mixed with various weight ratios, and prepared 300 images as the training dataset. In the purification process of synthesized organic compounds, powders such as silica gel, alumina, and celite are often used as adsorbents (**Figure 3-25a**). I thought that this system can automatically check the amount of the target compound remaining in the used adsorbents.

When preparing samples, the mixing ratio of silica gel/alumina/Celite was set randomly regardless of the mixing ratio of naphthalene (see **Figure 3-41** in Section 3.10.). To verify whether quantification was possible for some of the crystals were buried in the powder, the mixed sample was arranged with the powder and crystals partially overlapping (**Figure 3-25b**). In model construction, only the mixing ratio of naphthalene was input, and the ratios of the other three components were not input. The weight ratio of naphthalene was assigned as the ground truth of each image, and the ML model was trained to predict the weight ratio of naphthalene. The observed-predicted plot of 100 independent test images showed that image-based ML can also be applied to multicomponent mixture prediction, with MAE, RMSE, and R^2 of 3.4%, 4.8%, and 0.97, for the prediction of the weight ratio of naphthalene, respectively (**Figure 3-25c**). Although the prediction accuracy slightly dropped in the area with naphthalene ratio was less than 20%, predictions accuracy was almost the same as that of sugar/dietary salt.

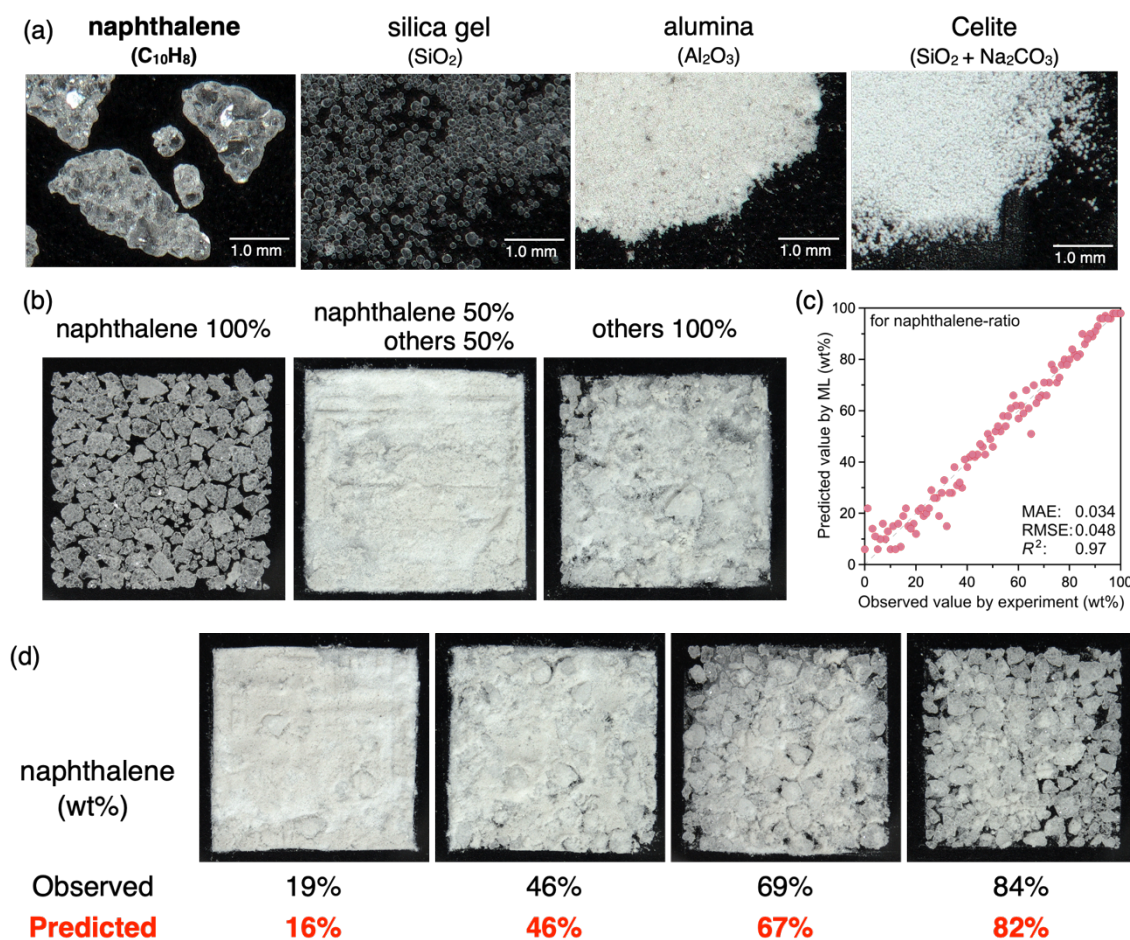


Figure 3-25. (a) Chemical formula and appearance of naphthalene and powder adsorbents. (b) Samples (200 mg) of digital-microscope images used for machine learning (others : silica gel/alumina/Celite mixture included at random ratios). (c) Observed-predicted plots for 100 test data (training data: 1% increment, 300 images). (d) Example of naphthalene weight ratio prediction under optimized conditions.

3.8. Prediction and Monitoring of Solid-State Reaction Yield

Based on the success to predict target component ratio from multi-component mixture in previous experiment, apply this image-based machine learning system to predict yields of real chemical reactions. *p*-Aminosalicylic acid (**PAS**) is an antibiotic used in the medical treatment of tuberculosis and is known to quantitatively convert *m*-aminophenol (**MAP**) via thermal decarboxylation in the solid state.^{19,20} This solid-state reaction results in continuous changes in its physical appearance as the reaction progresses. When fine powder **PAS** was heated, crystalline **MAP** gradually appears (**Figure 3-26a**). This reaction is known to proceed almost quantitatively.²¹

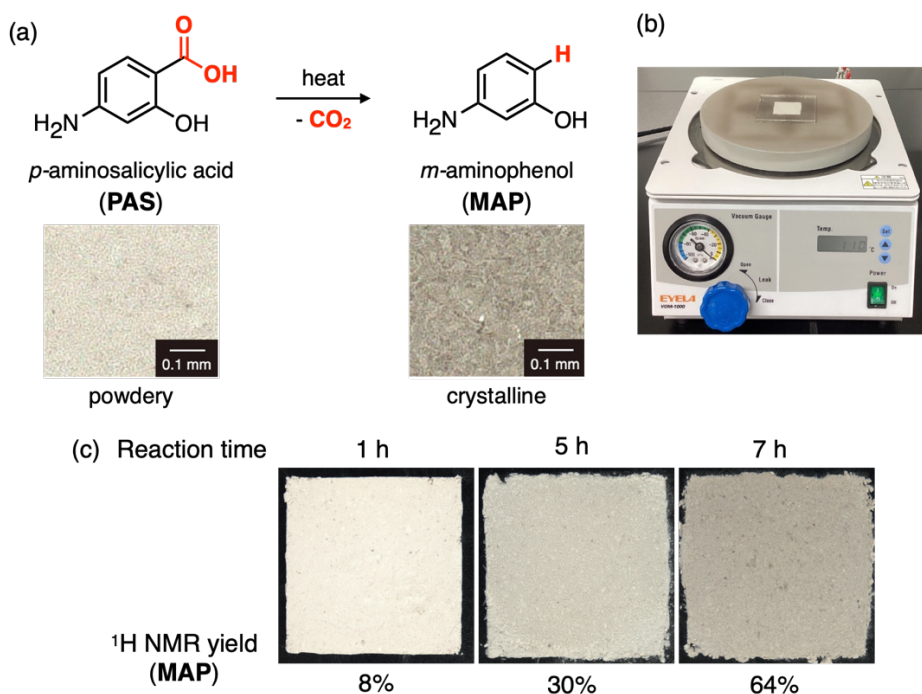


Figure 3-26. (a) Solid-state decarboxylation reaction of *p*-aminosalicylic acid (**PAS**). (b) Setting the solid-phase reaction of **PAS** using a hot plate at 110°C. (c) Example of sample image after reaction used for machine learning.

Image-taking conditions were followed as used in the previous experiment. 200 mg of **PAS** powder was spread flat over a 2.4 cm × 2.4 cm square area on a glass plate and sandwiched between two glass plates. After heating at 110°C on a hot plate (**Figure 3-26b**) for 0-48 hours, the heated sample were left at room temperature and photographed with a digital microscope (**Figure 3-26c**). As the reaction progresses, the sample crystallizes and turns brown, but because the reaction occurs heterogeneously due to heat transfer, it is difficult to simply predict the yield based on color alone. ¹H NMR (DMSO-

*d*₆) spectrum showed that no impurities were observed even after the reaction was complete. (See **Figure 3-42** in Section 3.10.)

Training datasets with different numbers of images (25, 50, and 75 images, **Figure 3-27**) were prepared. Data augmentation, test-time augmentation, and other machine learning settings were applied under optimized conditions. The prediction accuracy was evaluated by the same another 10 sample images. When 25 images were used as training data, prediction was failed completely, predicted yields were almost the same regardless of true NMR yield. (**Figure 3-28**). ML model constructed by 50 training images showed a better prediction score (MAE = 5.6%, RMSE = 7.5%, $R^2 = 0.95$). Since no effective improvement in the evaluation was observed between 50 and 75 training images, the optimal number of training images was set to 75 images (MAE = 5.7%, RMSE = 6.8%, $R^2 = 0.96$). Example of prediction result was shown in **Figure 3-29**. From these results, machine learning system enables nondestructive analysis of a solid-state chemical reaction.

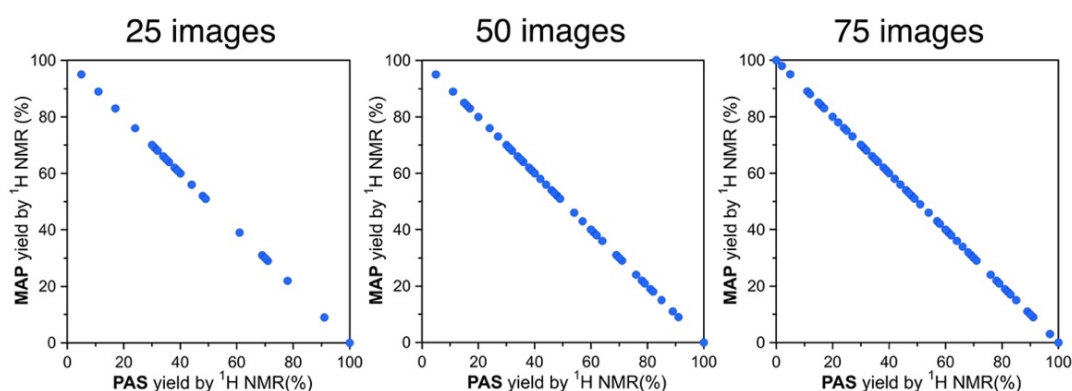


Figure 3-27. Distributions of PAS and MAP yields in the training data.

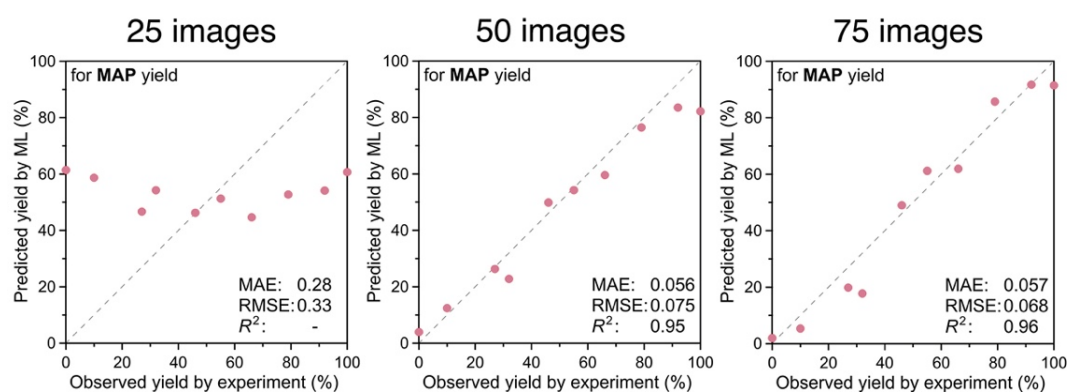


Figure 3-28. Observed–predicted plots of MAP yield for 10 test images using different numbers of training images.

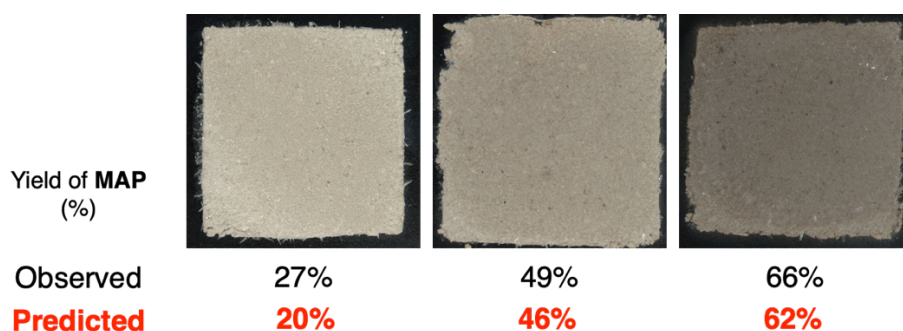


Figure 3-29. Yield prediction examples for 110°C (Training data : 50 images).

For next step, predict reaction yield under reaction temperature different from training data. PAS reaction was performed at 80°C or 100°C instead of 110°C, and sample images after reaction were given as test dataset for analysis. Five samples were created for each temperature with different reaction times and analyzed using both NMR and image machine learning. The change in yield by ^1H NMR (**Figure 3-30**) is consistent with this reaction being a first-order reaction.²⁰

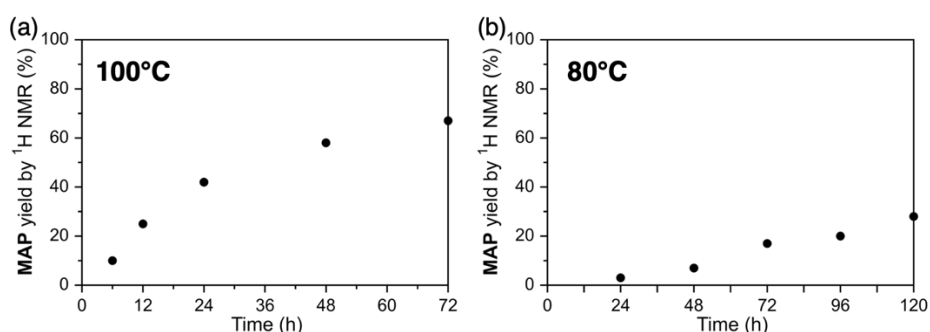


Figure 3-30. Plots of MAP reaction yield calculated from ^1H NMR spectra after heating PAS on a hot plate at (a) 100°C for 0-72 h and (b) 80°C for 0-120 h.

The results of predictions using ML are shown in **Figure 3-31**. For samples heated at 80°C and 100°C, yield predictions were achieved with errors of 12% and 8%, respectively, demonstrating that analysis is possible even for experimental conditions that were not provided as training data.

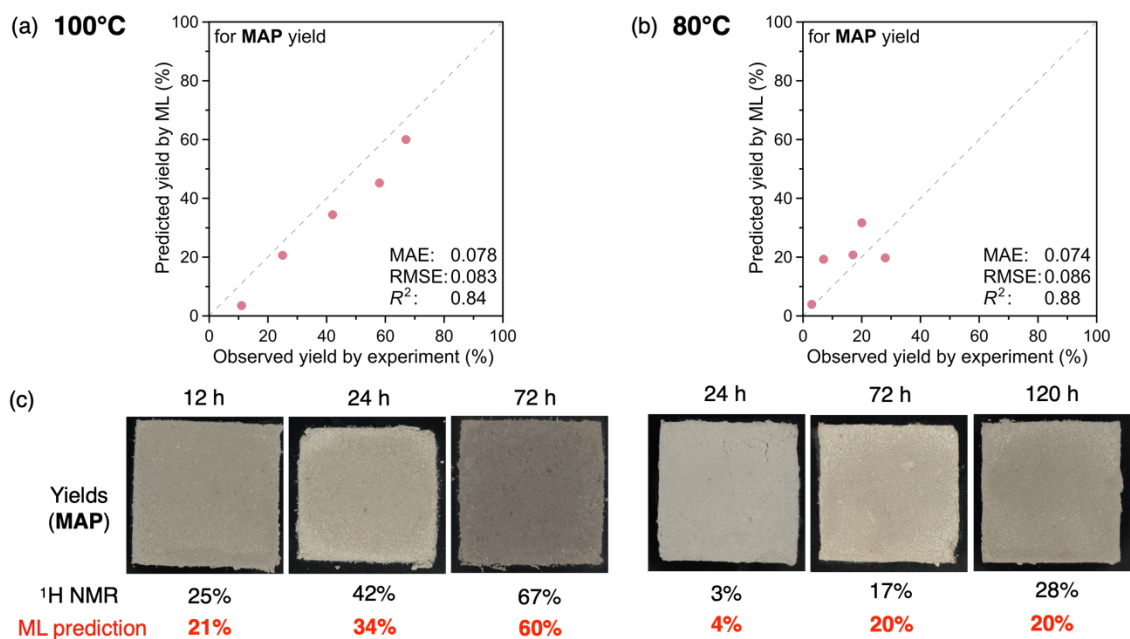


Figure 3-31. MAP reaction yield predictions for (a) 100°C for 0-72 h and (b) 80°C for 0-120 h. (c) Prediction examples for 100°C and 80°C.

Next, examined a slightly more complicated temperature profile for machine-learning based reaction analysis. **PAS** was first heated at 110°C for 3 h. Then, heating was stopped and left at room temperature for 3 h and then reheated at 110°C for a further 3 h. I took five different images of reaction mixtures each at 1 h and analyzed using ML model. **Figure 3-32a** shows ^1H NMR yields for samples continuously heated at 110°C for 1 to 6 h. Under this reaction condition, the yields increased almost monotonically with reaction time, which is good agreement with the reported trend of yield and reaction time.²¹ **Figure 3-32b** shows the yield change predictions by machine learning. It shows that the predicted yield increases by about 5.3% per 1 h during heating 110°C, while almost constant yield under room temperature. The predicted increase in yield was close to the average value (6.4%/h) calculated from the ^1H NMR yield, indicating that image-based machine learning is effective for predicting approximate reaction yields.

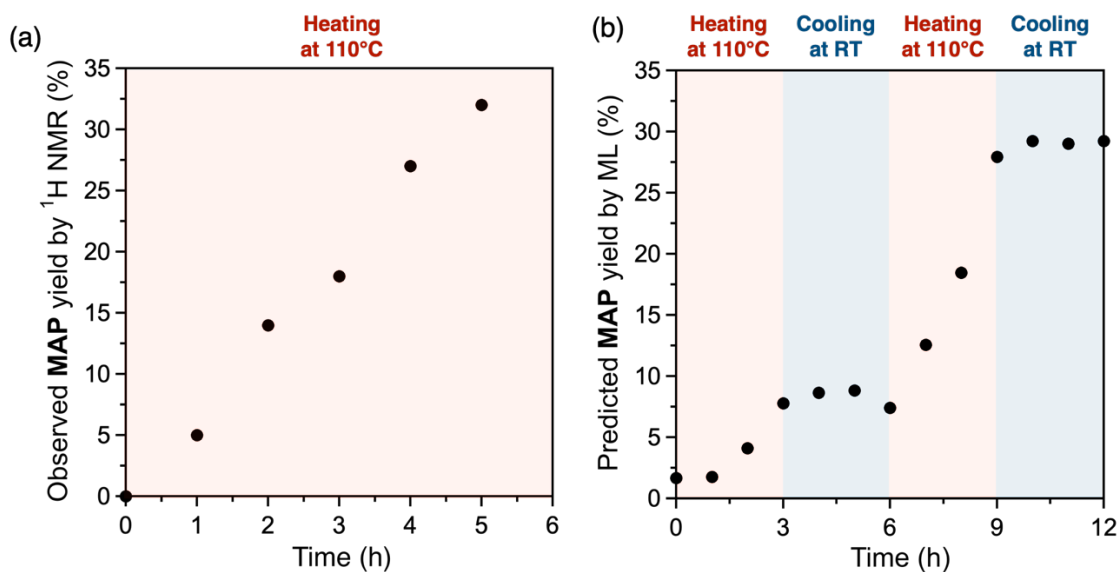


Figure 3-32. (a) ^1H NMR yield for continuous heating reaction for 1-6 hours. (b) Reaction monitoring by ML based yield prediction system under discontinuous heating conditions. Images of the reaction mixtures were taken every 1 h, and the average yields from five images at each time were used as the predicted MAP yield values showed in the graph.

Although NMR spectroscopy is more accurate than ML-based analysis, each analytical step requires sample preparation. For example, a portion of the sample needs to be taken and dissolved in a deuterated solvent. In this sense, ML analysis is easier and non-destructive, since image data can be acquired during the reaction without touching the sample. This machine learning system can therefore be useful for the quality control of pharmaceutical compounds that may deteriorate during storage.

I also attempted to make predictions from mobile camera images, to make it easier to obtain training data. After the reaction, the samples were photographed with a mobile camera (iPod) parallel to the hot plate at a distance of 8 cm (**Figure 3-33a**). Obtained image data were cropped to 1536×1536 pixel squares and used as training data (**Figure 3-33b**). The model constructed with 50 images of training data (same as digital microscope condition) can predict the MAP yield of 10 samples of test data with MAE = 6.3%, RMSE = 9.5%, and $R^2 = 0.89$ (**Figure 3-33c**). Although prediction accuracy was slightly lower than that of the digital microscope, mobile camera images could be apply as a more versatile prediction method.

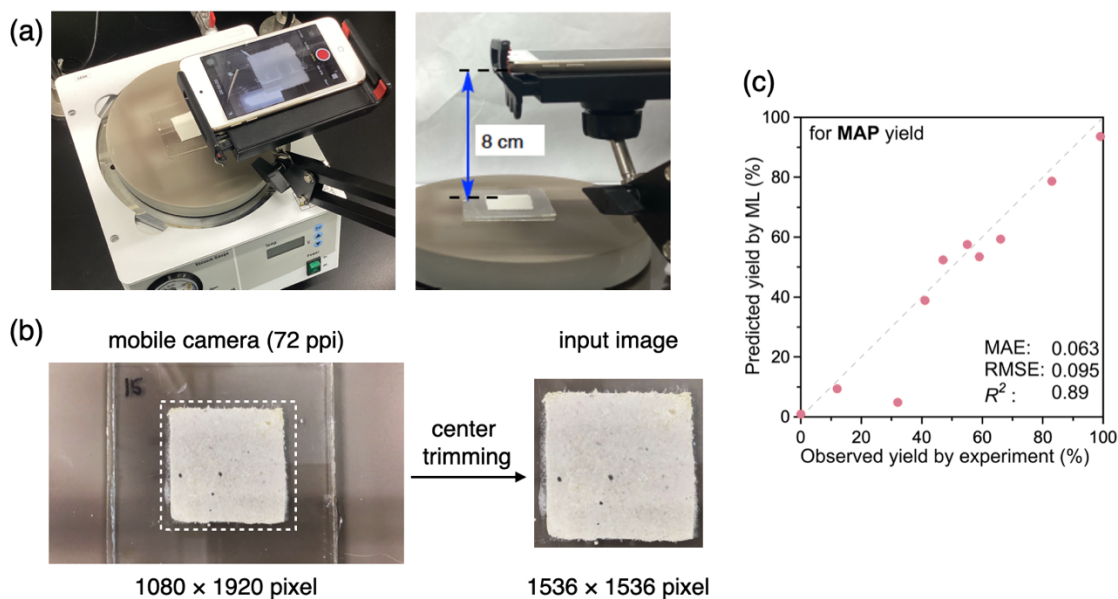


Figure 3-33. (a) Image acquisition method by mobile camera. (b) Original and trimmed images for image-based machine learning system. (c) Observed-predict plots for 10 test data constructed by 50 sample of mobile camera images.

Although these usefulness, conventional image-based ML methods require at least several dozen samples to be prepared as training data and photographed for each, which is a major obstacle to practicality. However, if the relationship between reaction time and yield is known, it would be possible to reduce time and cost to prepare training data by acquiring the reaction process by mobile camera and extracted images from the video as training data.

200 mg **PAS** sample was continuously heated for 48 hours (97% final NMR yield), and the video was captured using the timelapse function of mobile camera (iPod). Images extracted from each frame of the resulting video were cropped to 1536×1536 pixel squares. In solid-state reactions, the procedures involved in fractionation can significantly change reaction conditions, so yield values tied to training dataset was estimated from the observed ^1H NMR yields under the same conditions. Training dataset images were selected so that the yields associated with them were equal. For example, 10 images were selected for each of the 10 ranges of yields 0~10%, 10~20%, ... and 90~100%, for 100 images of training data (**Figure 3-34**).

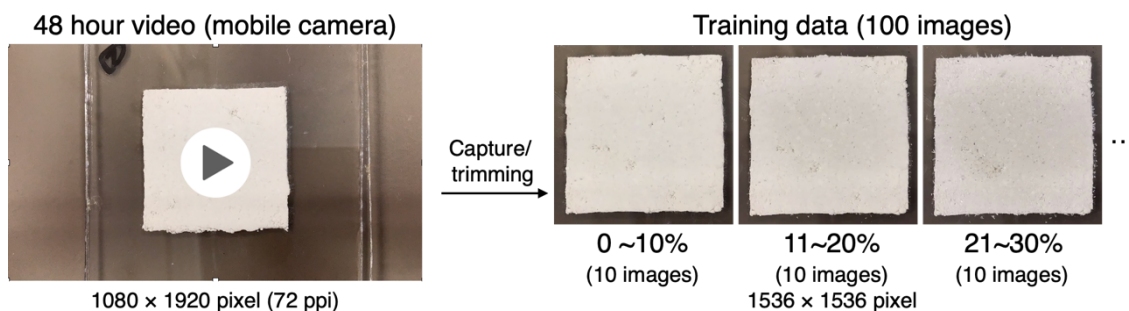


Figure 3-34. Method for acquiring training dataset from mobile camera.

I constructed model with training data (100-1000 images) extracted from a single timelapse video, try to predict yields for 30 samples of the same test data. (For test data, ^1H NMR yields were measured directly for each test data samples and used as true values.) As a result of increasing or decreasing the number of training data, accuracy reached its maximum (MAE = 11%, RMSE = 13%, $R^2 = 0.79$) when 500 images of training data were used (**Figure 3-35**). This result indicates that if the yield transition is known, a large amount of training data can be simultaneously obtained and used for modeling by using videos of reactions taken continuously.

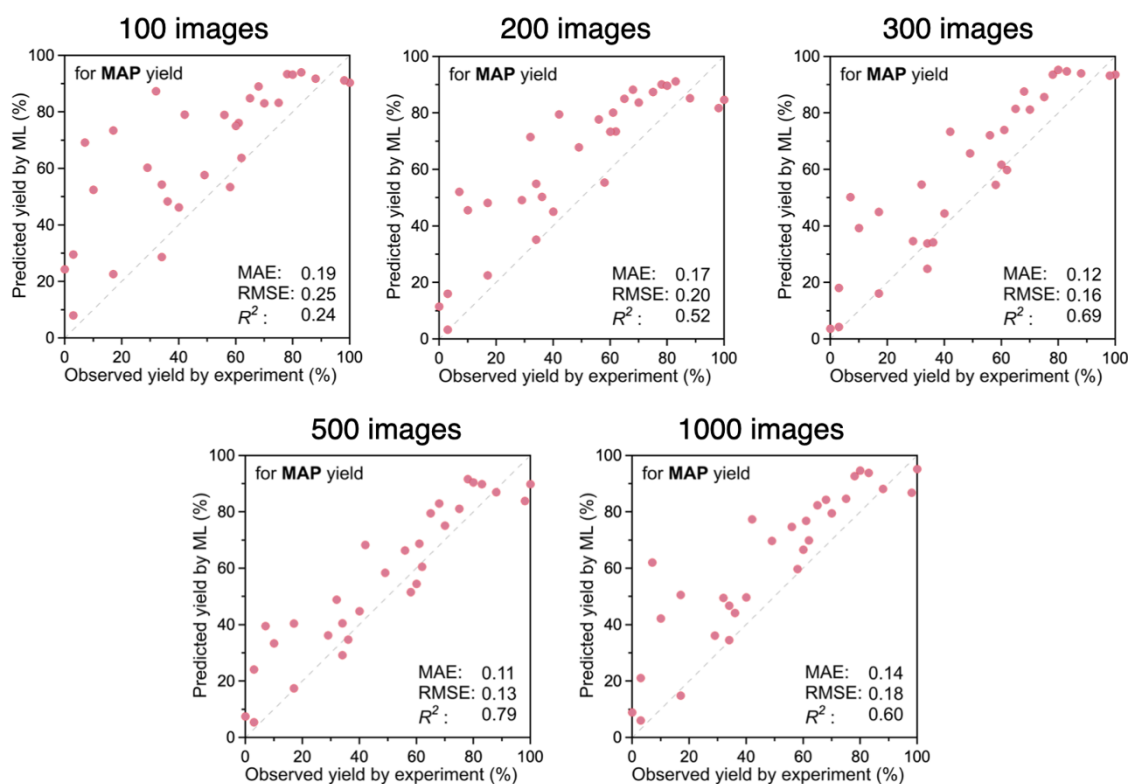


Figure 3-35. Observed–predicted plots of yields of **MAP** for test data (30 images) with model construct from 100/200/300/500/1000 images of clipped images.

By reducing bias and overfitting while keeping convenience of obtaining training data, use images extracted from multiple videos to improve prediction accuracy. Prepared two time-lapse videos independently and construct model by providing the same number of extracted images from each video as training data. Compared yield prediction accuracy for same 30 samples of test data, the model construct by 400 training data (**Figure 3-36a**, 200 images each extracted from two videos) was higher accuracy (MAE = 9.6%, RMSE = 12%, $R^2 = 0.83$) than previous model (500 images from single video, **Figure 3-35**).

Similarly, the model constructed from three reaction videos was found to have even higher accuracy (MAE = 8.2%, RMSE = 10%, $R^2 = 0.87$, **Figure 3-36b, 37**) with 600 images of training data (200 images were extracted per video). These results indicate that multiple samples can be used to achieve both versatility in prediction and simplicity in data collection.

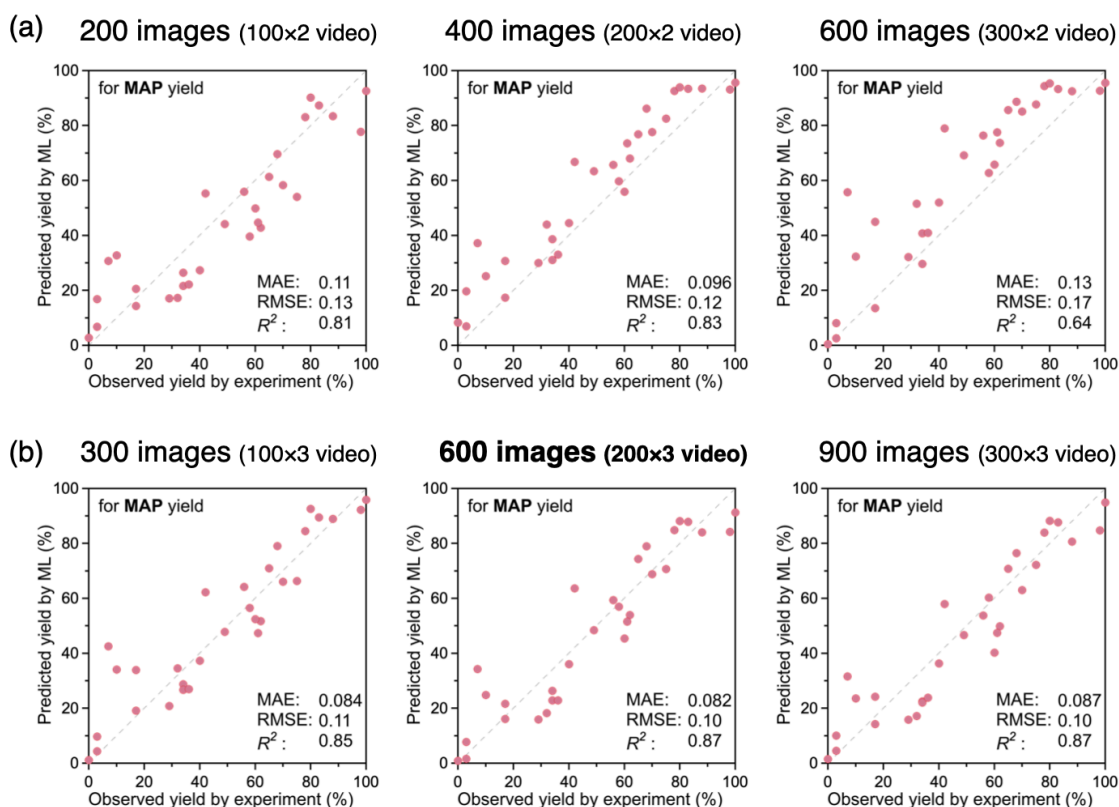


Figure 3-36. Observed–predicted plots of yields of **MAP** for test data (30 images) with model construct from (a) 100/200/300 images clipped from each two video. (b) 100/200/300 images clipped from each three video.

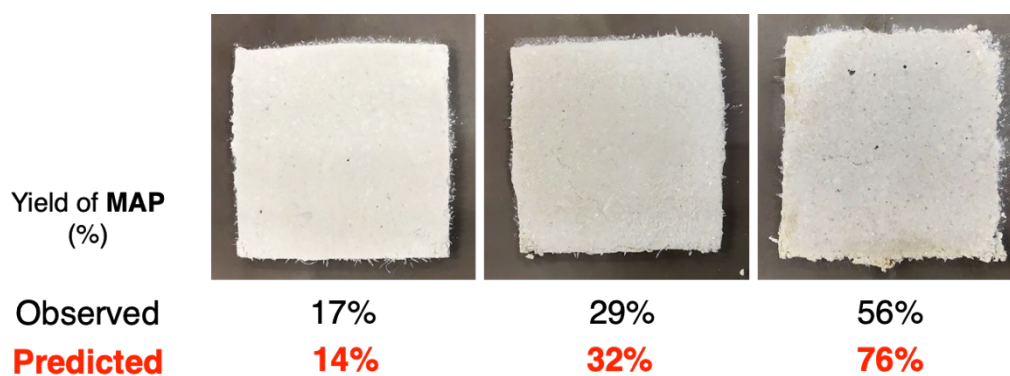


Figure 3-37. Yield prediction examples by optimized model (600 images, for **Figure 3-36b**).

3.9. Conclusion

The author developed an image-based ML mixing-ratio for predicting sugar/dietary salt mixing ratios. First, the hypothesis showed at the beginning of this chapter was proved by image-based ML constructed from 100 training dataset. Optimization results showed improved prediction accuracy of MAE = 3.9% and RMSE = 6.6% using 300 images, which is higher prediction accuracy than visual observation. The usefulness of system was demonstrated in terms of the speed of decision making, which can predict quickly (a few seconds per 10 images) even for large amounts of test data, and reproducibility. The ability to analyze remotely using mobile camera and Google Colaboratory system can system more simple and rapid predictive tool for actual reaction mixtures. Image-based ML is also applicable to more complex mixtures containing four components mixture and nondestructive reaction analysis of solid-state decarboxylation of **PAS**. A ML model constructed from 300 images of α -glycine and γ -glycine was able to predict polymorph ratios from the images with an error of 4.0%. It is also possible to predict the enantiomeric ratio of D-/L- tartaric acid, which is difficult to distinguish between crystals with the naked eye. Furthermore, by inputting images of the reaction mixture and ^1H NMR yields for each sample, the reaction yields were successfully predicted. **MAP** yield in solid-state decarboxylation of **PAS** was predicted with an error of 5.7%. If the relationship between reaction time and yield is known, succeeded in predicting yields within an average error of 10% based on training data obtained from several experiments by using images clipped from a series of video recorded the reaction process. These results indicate the applicability of image-based ML to various chemical reaction analysis. After submitted as academic paper,²² this research attracted attention of media and was featured in newspapers and other publications (**Figure 3-38**).²³⁻³⁰



Figure 3-38. Media coverages for newspapers, online news, and press release.

3.10. Experimental Section

Reagents and equipment

Sugar and dietary salt were purchased from local supermarkets (sugar : AEON Co., Ltd., dietary salt : Salt Industry Center of Japan) and used without further purification. Glycine was purchased from FUJIFILM WAKO Pure Chemical Industries and recrystallized as α - and γ -glycine by the reported procedure. The rest of the compounds used were of analytical grade and purchased from FUJIFILM WAKO Pure Chemical Industries, TCI Co., Ltd., or Kanto Chemical Co., Inc., and they were used without further purification.

All of photographs for machine learning (ML) were taken by a KEYENCE VHX-970F digital microscope (image resolution: 2048×1536 pixels, 96 ppi), and trimmed to a square shape of 1536×1536 pixels, unless otherwise noted. Mobile camera pictures were taken with an iPod Touch (7th generation, Apple Inc.)

Image data

To prepare images of sugar/dietary salt mixtures for training and test datasets, each sample was weighed on a glass plate. Weighed samples were evenly mixed with a spatula to ensure uniformity of images. Well-mixed samples were then arranged in a square shape ($2.4 \text{ cm} \times 2.4 \text{ cm}$) with thickness of less than 1.0 mm. For digital microscope images, magnification and background color were set to $10\times$ and black, respectively. Other condition settings were optimized as explained in main text. For each mixture, one image was taken (2048×1536 pixels, 96 ppi) and saved in .tiff file format. The collected images were trimmed to a square shape (1536×1536 pixels, 96 ppi) and compressed as a jpeg image by Adobe Photoshop. A dataset of specified number of images was compiled with a text file describing the actual mixing ratio. Image collection of the other examples was performed according to the conditions optimized with sugar/dietary salt mixture.

In the case of image collection using a mobile camera, an iPod Touch (2448×3264 pixels, 72 ppi), the images were taken at distance of 8.2 cm from the mixture sample with the magnification of $1.5\times$. The collected images were trimmed to be 1536×1536 pixels square to accommodate the previous image format.

Machine learning

All ML model was developed using PyTorch. It was trained for 120 epochs in total with the Adam optimizer. For the first 20 epochs, only the head regressor was trained with the ImageNet-pretrained convolutional neural networks (CNN) from the timm library. For the latter 100 epochs, all parameters of both CNN and head regressor were trained. The learning rate (lr) was set to $lr = 1e-5$ for the first warmup phase with OneCycleLR scheduler with $max_lr = 1e-3$, and to $lr = 1e-7$ for the second uptraining phase with OneCycleLR scheduler with $max_lr = 5e-4$. The batch size was 16 as default, and 8 was only used when the number of training samples was less than 50. For the loss function, the soft cross-entropy defined by $-\sum_{i=1}^N y_i \log \hat{y}_i$ for N predicted ratios $\hat{y}_i$ and true ratios y_i was used because the $[0,1]$ -bounded values $0 \leq \hat{y}_i \leq 1$ and $0 \leq y_i \leq 1$ were compared. Only for iPod fine-tuning case, 50 epochs were set for the warm-up phase. For the extremely-small-sample problem of iPod fine-tuning and 10-sample training, no validation set was taken and the final model after the specified epochs was directly used for evaluation. Except for these two cases, a random 10% of the training set was used as the validation set, and the best model with respect to the prediction performance for the validation set was selected for the test-set evaluation.

The Fourier-transform infrared (FT-IR) spectra were measured with a JASCO Co. FT/IR-4600 spectrometer. The powder X-ray diffraction (PXRD) spectra were measured with a Rigaku SmartLab X-ray diffractometer (CuK_{α} radiation $\lambda = 1.54184 \text{ \AA}$) equipped with a HyPix-3000 photon counting detector. All 1H NMR spectra were recorded using JEOL JNM-ECZ400S spectrometer and chemical shifts were reported in parts per million (ppm) relative to an internal standard tetramethylsilane ($\delta = 0.00$ ppm for 1H NMR in $DMSO-d_6$). All of the photographs for machine learning were taken by a KEYENCE VHX-970F digital microscope (image resolution: 2048×1536 pixels, 96 ppi), and trimmed to a square shape of 1536×1536 pixels, unless otherwise noted. The solid-state heating reaction of *p*-aminosalicylic acid (**PAS**) was performed on a hot plate (EYELA VOM-1000). The mobile camera pictures were taken with an iPod Touch (7th generation, Apple Inc.).

Recrystallization of glycine

Recrystallization of glycine was conducted by a reported procedure.¹²⁻¹⁴

Recrystallization of α -glycine

Glycine (500 g) and distilled water (2.0 L) were added to a 2 L beaker with a magnetic stirrer, and the solution was warmed to 33°C. After complete dissolution of glycine, the solution was filtered to remove the residual solid. The filtrate was transferred to a 2 L beaker with a magnetic stirrer and kept at 33°C for 15 min. Subsequently, the beaker was cooled using a water/ice bath with stirring. After 1 h, the precipitated crystals were collected from the solution by suction filtration. Vacuum drying of the crystals at room temperature overnight yielded α -glycine crystals (169 g) in 34% yield.

Recrystallization of γ -glycine

Glycine (500 g) and 1 M HCl aq. (1.3 L) were added to a 2 L beaker with a magnetic stirrer, and the solution was warmed to 33°C. After complete dissolution of glycine, the solution was filtered to remove the residual solid. The filtrate was transferred to a 2 L beaker with a magnetic stirrer and kept at 33°C for 15 min. Subsequently, the beaker was cooled using a water/ice bath with stirring. After 1 h, the precipitated crystals were collected from the solution by suction filtration. Vacuum drying of the crystals at room temperature overnight yielded γ -glycine crystals (165 g) in 33% yield.

Characterization of α - and γ -glycine

Recrystallized α - and γ -glycine were confirmed by FT-IR and PXRD. The FT-IR spectra (Figure 3-39) and PXRD patterns (Figure 3-40) observed of α - and γ -glycine were in good agreement with the references.¹²⁻¹⁴

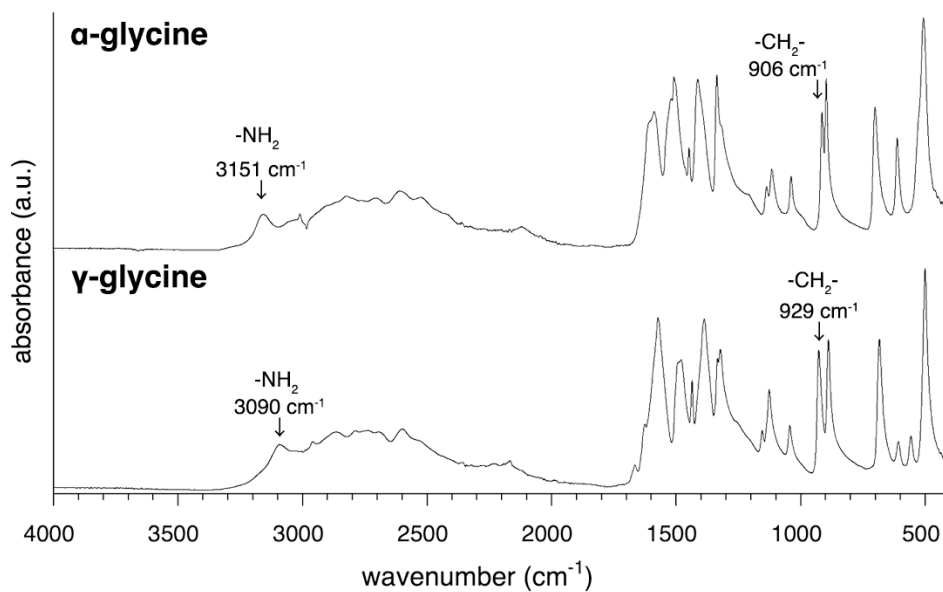


Figure 3-39. FT-IR spectra of recrystallized α - and γ -glycine (ATR, neat).

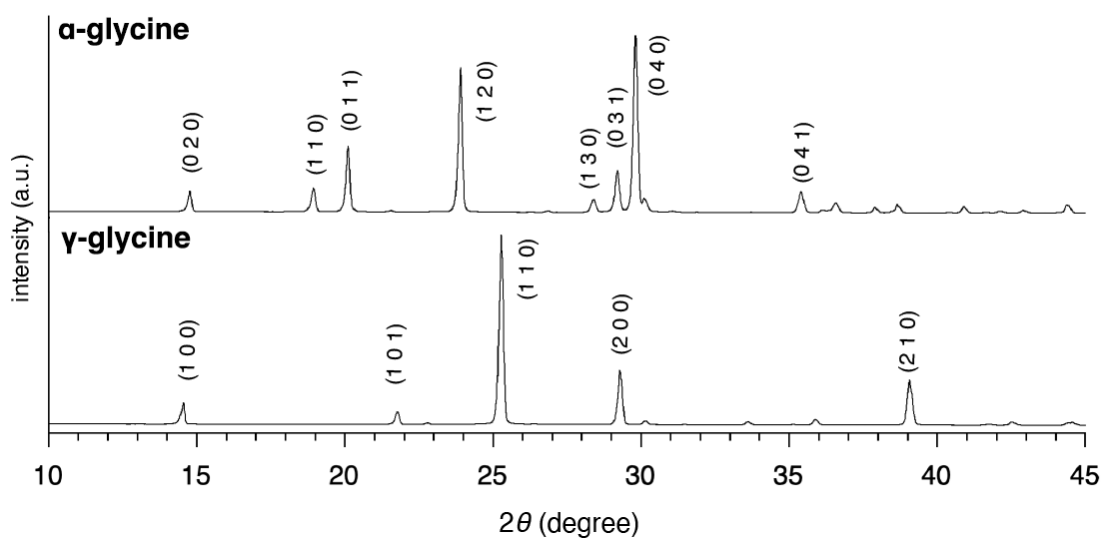


Figure 3-40. PXRD patterns of recrystallized α - and γ -glycine.

Dataset settings in four-component systems

As mentioned in Section 3.4., the silica gel/alumina/Cellite mixing ratio was set randomly. Silica gel/alumina/Cellite mixing ratios for the training data (300 images) and test data (100 images) are shown in **Figure 3-41**.

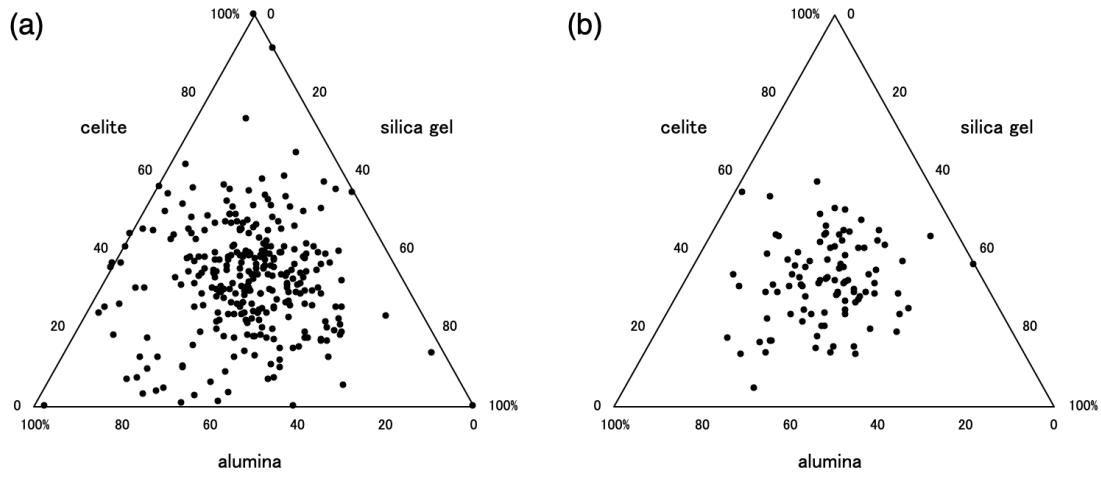


Figure 3-41. Mixing ratio of silica gel : alumina : Cellite for (a) training and (b) test dataset.

Experiment for *p*-aminosalicylic acid decarboxylation reaction

200 mg of *p*-aminosalicylic acid (**PAS**) powder was spread over a 2.4 cm × 2.4 cm square area on a glass plate and sandwiched between two glass plates. After heating on a hot plate at 110°C for a predetermined time at 0–48 hours, heated samples were stand at room temperature and photographed with a digital microscope. The training/test dataset that indicates the reaction yield of *m*-aminophenol (**MAP**) obtained by decarboxylation reaction utilized NMR yield calculated from proton signal integration ratios in good agreement with the references (**Figure 3-42**).^{20,21,31,32}

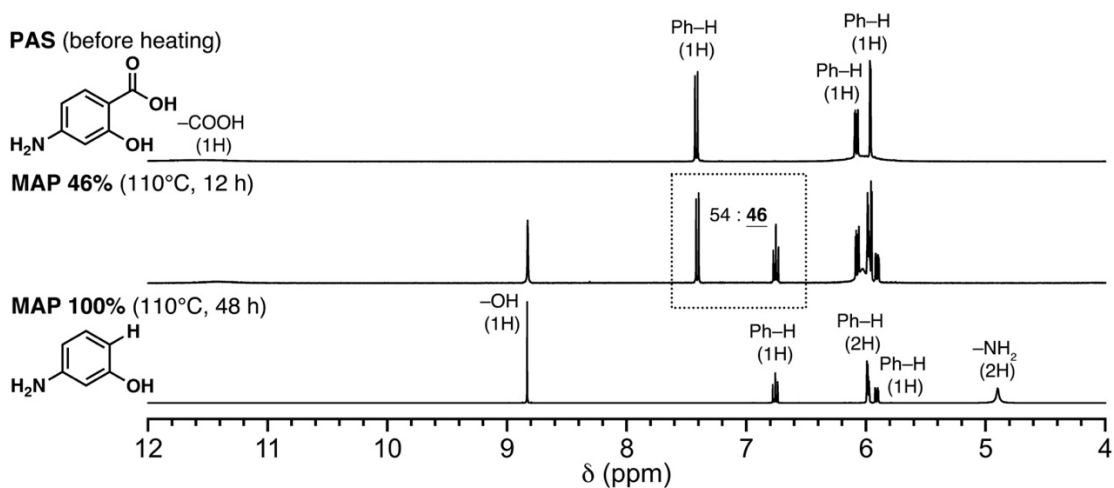


Figure 3-42. ¹H NMR spectra (DMSO-*d*₆) of **PAS**, reaction mixture (**PAS** : **MAP** = 54 : 46), and obtained **MAP**.

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Chapter 4

Applied to Chemical Research:

Mixing Ratio Prediction in Trace Samples and

Application to Metal Ion Sensor Molecules

4.1. Introduction

In this chapter, apply image-based ML system to chemical research for improve efficiency and molecular performance. ML conditions used in previous studies (optimized in Chapter 3) have some problems in terms of practicality in chemical research. First, at least 10 g of target compounds is needed to construct models, because 200 mg samples were used per image. Generally, instrumental analysis can be performed by at least 1 mg of sample. If image-based ML could make predictions from mg-scale solids, practical use would be improved. Secondly, if the appearance of the solids differs from that of the training data due to differences in synthesis/crystallization methods, prediction accuracy can be significantly decreased, even for chemically pure and identical substances. There remain issues to be addressed in terms of scale and reproducibility in order to use image-based ML method. As practical applications, I attempted chirality prediction from solid dried from solution, and improved detection ability of isopyrazole metal ion sensor synthesized in Chapter 2.

I tried to distinct (*R*)- or (*S*)- 1,1'-binaphthyl from solids by image-based ML. 1,1'-Binaphthyl has axial chirality because steric hindrance prevents free rotation around the C-C single bond at room temperature.¹ When 1,1'-binaphthyl is heated above its melting point (approximately 160°C), C-C single bond becomes free to rotate and rapidly racemizes. Chirality of 1,1'-binaphthyl can be randomly biased by heating above its melting point, keep in a supercooled state (150°C), and solidified by drop dry ice. Chiral crystals of 1,1'-binaphthyl can be efficiently obtained with a probability of *R* : *S* = 1 : 1 by stirring supercooled melt (**Figure 4-1**).² This spontaneous resolution involved only pure 1,1'-binaphthyl.

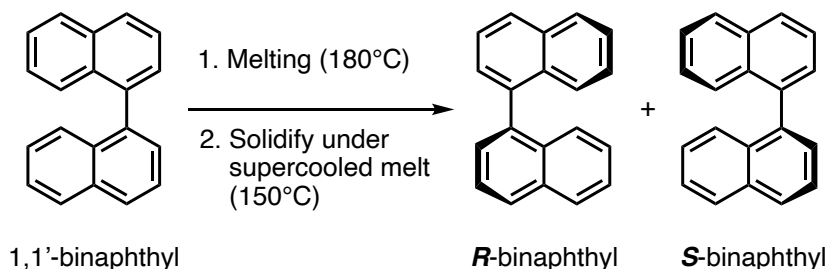


Figure 4-1. Spontaneous resolution of 1,1'-binaphthyl enantiomers.

1,1'-binaphthyl have different crystal structure by recrystallization from acetone solution depends on optical purity.³ Chiral and racemic crystals of 1,1'-binaphthyl recrystallized from acetone are tetragonal-bipyramidal ($P4_12_12$) and monoclinic ($C2/c$), according to single-crystal X-ray diffraction analysis.⁴

The probability of chirality can be influenced by the presence of chiral compounds. *R*-1,1'-binaphthyl tends to be obtained when chiral molecules such as L-alanine, L-proline, and *R*-1,1'-bi-2-naphthol (*R*-BINOL) exist for 5~10% weight ratios.⁵ However, the mechanism or rules has not yet been completely clarified. ML prediction might reduce time and effort required for screening to elucidate these principles.

Analysis of molecular chirality is important for elucidating biological activity and optical properties of molecule as it relates to pharmacological and functional molecules.⁶ In general, enantiomer ratio and enantiomeric excess (*e.e.*) are determined by polarimetry, circular dichroism (CD) spectroscopy,⁷ and chiral high-performance liquid chromatography (HPLC),⁸ based on polarized absorption or interaction differences with the chiral stationary phase. However, identifying chirality for polarimetry requires specific helical information of target compound accurate preparation of the solution. For some compounds, specific rotation values or circular dichroism signal are too weak to be detected even can dramatically affect selectivity of asymmetric reactions.^{9,10} Finding suitable separation conditions in chiral HPLC is not always easy, and chiral separations generally take at least several minutes per sample. Hence, chiral screening for large amounts of samples is both time-consuming and labor-intensive. On the other hand, chirality can sometimes be identified by differences in shape. As mentioned in Chapter 3, Louis Pasteur once succeeded in identifying D-/L-tartrate from differences in macroscopic crystal shapes. Among 230 space groups, chiral molecules are always obtained as 65 Sohncke space groups,^{11,12} which contain only symmetry operations of the first kind (rotations and translations) with absence symmetry operations of the second kind (mirror planes and inversion centers). Even achiral molecules can have chirality in crystalline state.¹³ Chirality can sometimes be identified not only from differences in shape but also from differences in polarization behavior, such as refraction and interference exposed to polarized light. For example, chiral crystal quartz (α -SiO₂) can be distinguished by the orientation of interference fringes called Airy's spiral.^{14,15} From these findings, it may be possible to distinguish enantiomers based on visual information obtained from bulk crystals using image-based ML.

I also tried to use image-based ML for improving performance of molecular sensor. As target molecule, isopyrazole metal ion sensor **1**, as author synthesized in Chapter 2 (**Figure 4-2**) was used.¹⁶ Development of new molecular sensors requires some processes like synthesis, characterization, and performance evaluation, but it is not always possible to synthesize sensors that are more quantitative or selective than those reported so far. This is one of the hurdles in the development of new molecular sensors.

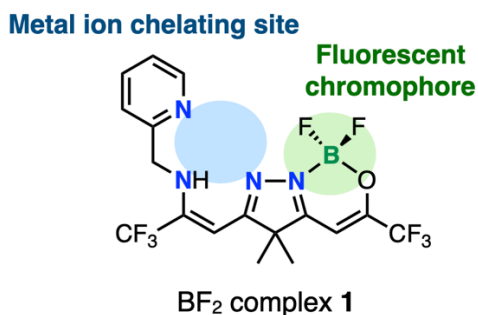


Figure 4-2. Structure and molecular design of isopyrazole fluorescent ion sensor **1**.

Several studies have been reported that combine fluorescent molecular sensors with machine learning to achieve higher performance. Detection limit of the molecular sensor was improved up to 100 times, by distinguish peaks of the target substance from noise signals in the fluorescence spectrum by ML.¹⁷ In other study, simultaneous quantification of two metal ions was achieved from their very similar fluorescence spectra.^{18,19} However, most of those ML analyses are based on spectral data obtained from instrumental analysis. There are only few examples of improved molecular sensor performance directly from image data of reaction mixtures, without required for analysis equipment.²⁰ In this chapter, I attempted to use image-based ML to improve quantitative performance from changes in the appearance of ion sensor **1**.

4.2. Predict Mixing Ratios from Dried Droplets by ML

As a preliminary study, our laboratory successfully predicted weight ratios from salicylic acid and acetylsalicylic acid from solid images, obtained by drying solution on glass plate (**Figure 4-3**). For prediction, mixture of salicylic acid and acetylsalicylic acid (total 100 mg) was completely dissolved in Et₂O (4 mL). Obtained solution (25 mg/mL) was taken with a pipette and one drop (0.05 mL, equivalent to solute approximately 1.25 mg) was gently dropped on a glass plate (**Figure 4-4**). After solution was completely evaporated (~5 minutes), sample was photographed with glass plate by digital microscope (magnification: ×20). ML model construction was carried out in the same way as described in Chapter 3.

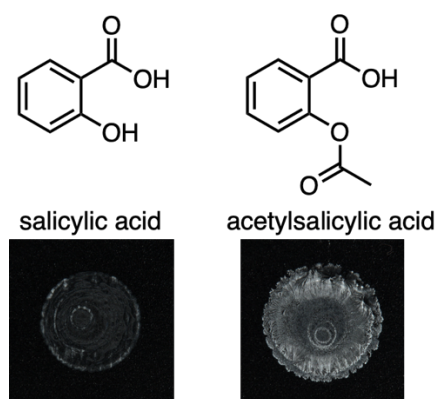


Figure 4-3. Structure and digital microscope images of salicylic acid and acetylsalicylic acid after natural drying.

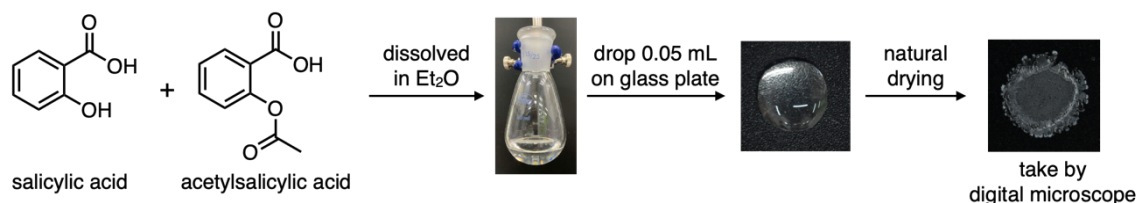


Figure 4-4. Schematic diagram of image collection method for salicylic acid/acetylsalicylic acid prediction.

ML model constructed 110 digital microscope images successfully predicted salicylic acid / acetylsalicylic acid weight ratio of 100 test data images, with mean absolute error (MAE) of 6.8% (**Figure 4-5, 4-6**). Although the accuracy was slightly reduced, prediction was still successful even when the concentration was lowered to 2.5 mg/mL. For a more dilute solution (0.25 mg/mL), prediction accuracy decreased significantly for samples with a high proportion of acetylsalicylic acid.

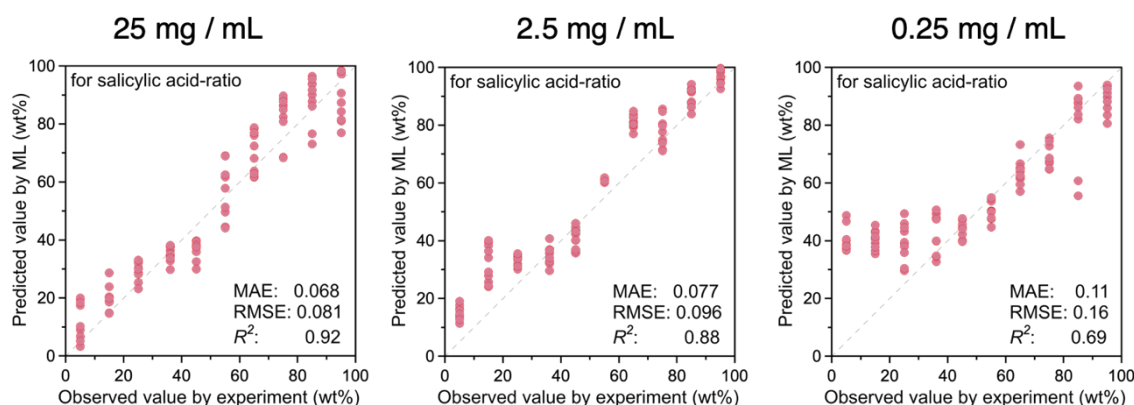


Figure 4-5. Observed-predicted plots for 100 test datasets for salicylic acid weight percent (25, 2.5, 0.25 mg/mL).

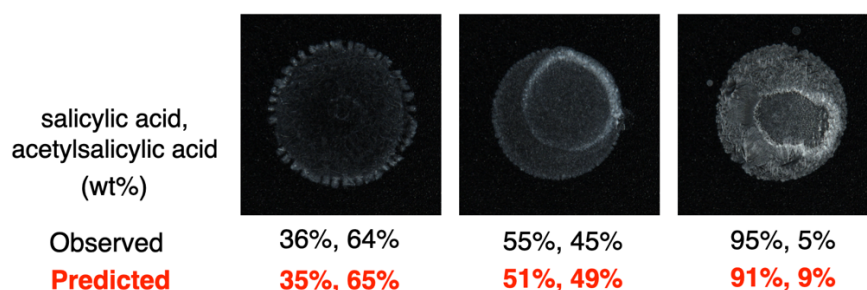


Figure 4-6. Prediction examples for salicylic acid weight percent. (concentration : 25 mg/mL).

Predicting mixing ratio from trace amounts of solids from droplets is significant because it reduces the amount of sample required for prediction, compared to the method using 200 mg of solid sample (predictions in Chapter 3). It is also useful as an efficient method for acquiring image data quickly from samples in solutions. For these reasons, I attempted to predict enantiomeric ratio of 1,1'-binaphthyl from solid images obtained by dried droplets onto a glass plate.

4.3. Predict Enantiomeric Ratio from Dried Droplets

Optimization of Solvent

I attempted to predict enantiomeric ratio of 1,1'-binaphthyl from solid images obtained by dried droplets onto a glass plate. As solvent, dichloromethane and benzene was selected by high solubility for 1,1'-binaphthyl and low boiling points suitable for spontaneous evaporation. Methods for creating training data was followed by salicylic acid/acetylsalicylic acid mixing ratio prediction. 50 mg/mL of 1,1'-binaphthyl solution was prepared, and 20 μ L (equivalent to approximately 1 mg of binaphthyl) was dropped onto a glass plate, and images were obtained after sufficient natural evaporation (**Figure 4-7**). For both solvents, 330 sample images were used as training data to construct ML models, and 100 sample images were given as test data to evaluate their performance. Enantiomeric ratio of 1,1'-binaphthyl was analyzed by chiral HPLC (See **Figure 4-30** in Chapter 4.9.).

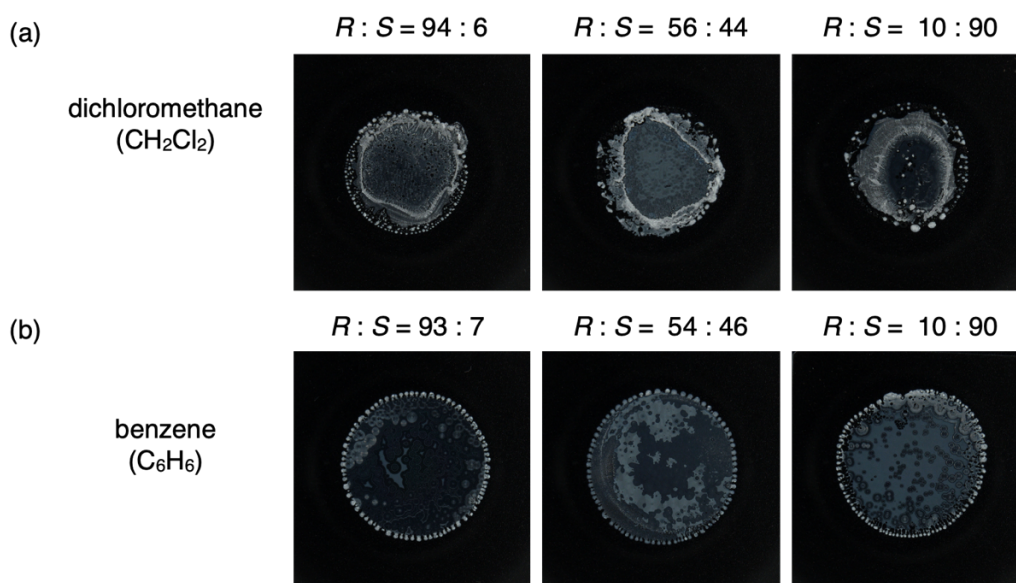


Figure 4-7. Digital-microscope images of 1,1'-binaphthyl droplet after natural evaporation on soda glass. (a) dichloromethane (CH₂Cl₂), (b) benzene (C₆H₆).

Enantiomeric ratios could not be predicted for dichloromethane, because predicted values were almost constant regardless of true enantiomeric ratios (**Figure 4-8a**). For benzene, observed-predicted plot showed an inverted V shape (**Figure 4-8b**). In other words, ML model can distinguish between racemic and chiral binaphthyl but failed to predict whether (*R*)- or (*S*)-binaphthyl is more abundant.

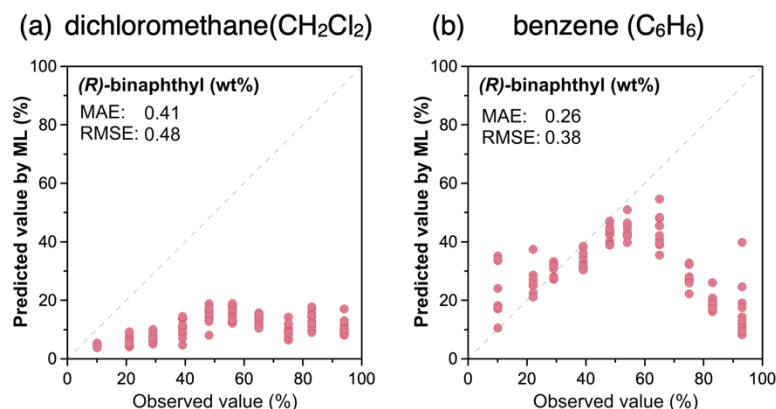


Figure 4-8. Observed-predicted plots for 100 test datasets for (*R*)-1,1'-binaphthyl weight percent. Horizontal axis shows measured enantiomeric ratio by HPLC, and vertical axis shows predicted values by ML (training data: 330 images).

When glass plate was replaced from soda glass to potassium glass, appearance solids was changed (**Figure 4-9**). ML models were constructed in the same manner, by changing number of sample images (training data) in the range of 110 to 550. For each model, evaluated the performance by 110 sample images predictions. There was almost no correlation between observed and predicted values by 330 training images or less. The model constructed 550 image of training data confirmed a strong correlation (MAE = 7.6 %, RMSE = 10%, and $R^2 = 0.86$) between observed and predicted values, indicating that this ML system is applicable to 1,1'-binaphthyl (**Figure 4-10**).

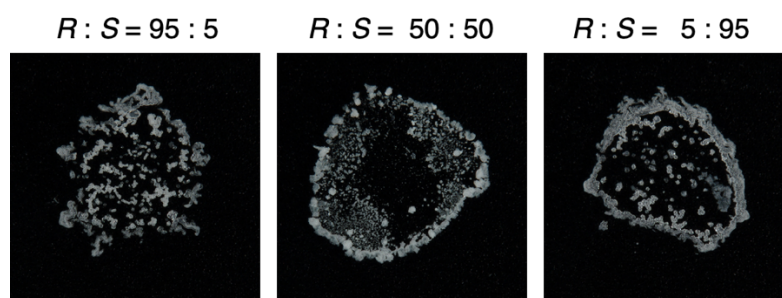


Figure 4-9. Digital-microscope images of 1,1'-binaphthyl droplet after natural evaporation on potassium glass.

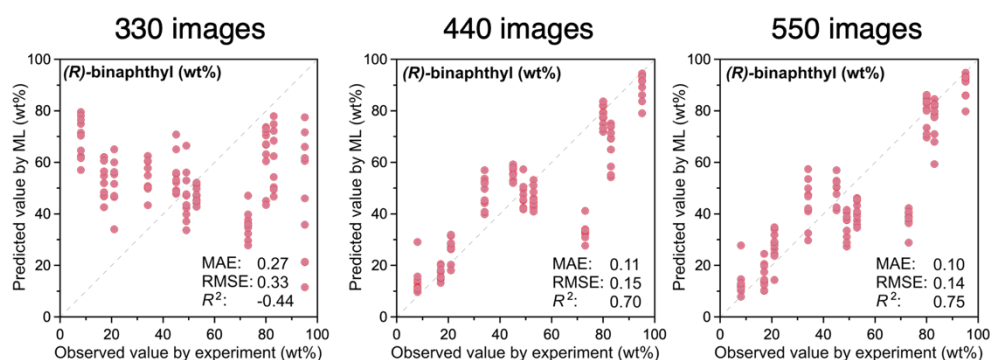


Figure 4-10. Observed-predicted plots for 100 test datasets for (*R*)-1,1'-binaphthyl weight percent under different amount of training data.

However, for created on a different day from the training data, accuracy of predictions for sample images was only about 70% at best (**Figure 4-11**), even after adjusting training data. It was found that solids shapes of 1,1'-binaphthyl were apparently different even for samples taken on the same date and time.

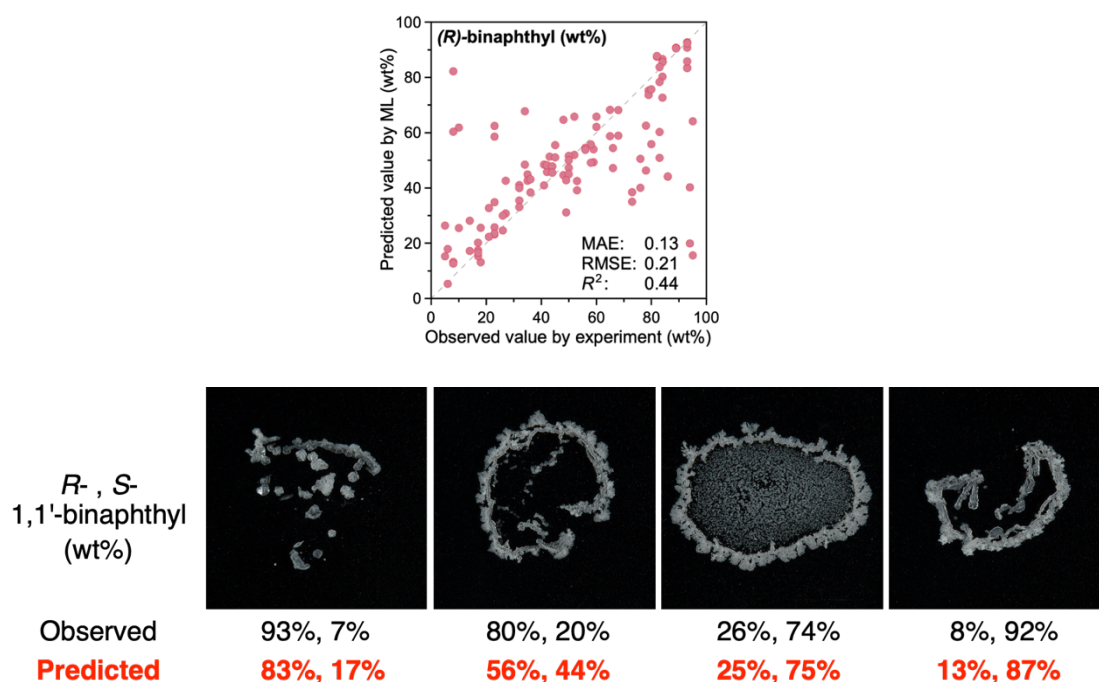


Figure 4-11. Observed-predicted plots for 100 test datasets and prediction examples for (*R*)-1,1'-binaphthyl weight percent (training data: 550 images).

I thought that it is important for shape reproducibility of 1,1'-binaphthyl to make image-based ML predictions more practical. I reviewed surface conditions for drying droplets.

Optimization of Surfaces

I searched for the best surface material for ML prediction. Appearance of 1,1'-binaphthyl solids obtained from drying and solidification varied greatly depending on the surface material including particle size and spreading, and these appearance differences may affect prediction accuracy. Materials that do not react, dissolve, or permeate with a benzene solution of 1,1'-binaphthyl at room temperature and allows the total amount of 1,1'-binaphthyl in solution to deposit on the solid surface is desirable. Four materials were selected to satisfy these conditions: polypropylene, aluminum, brass, and Teflon plates (Figure 4-12).

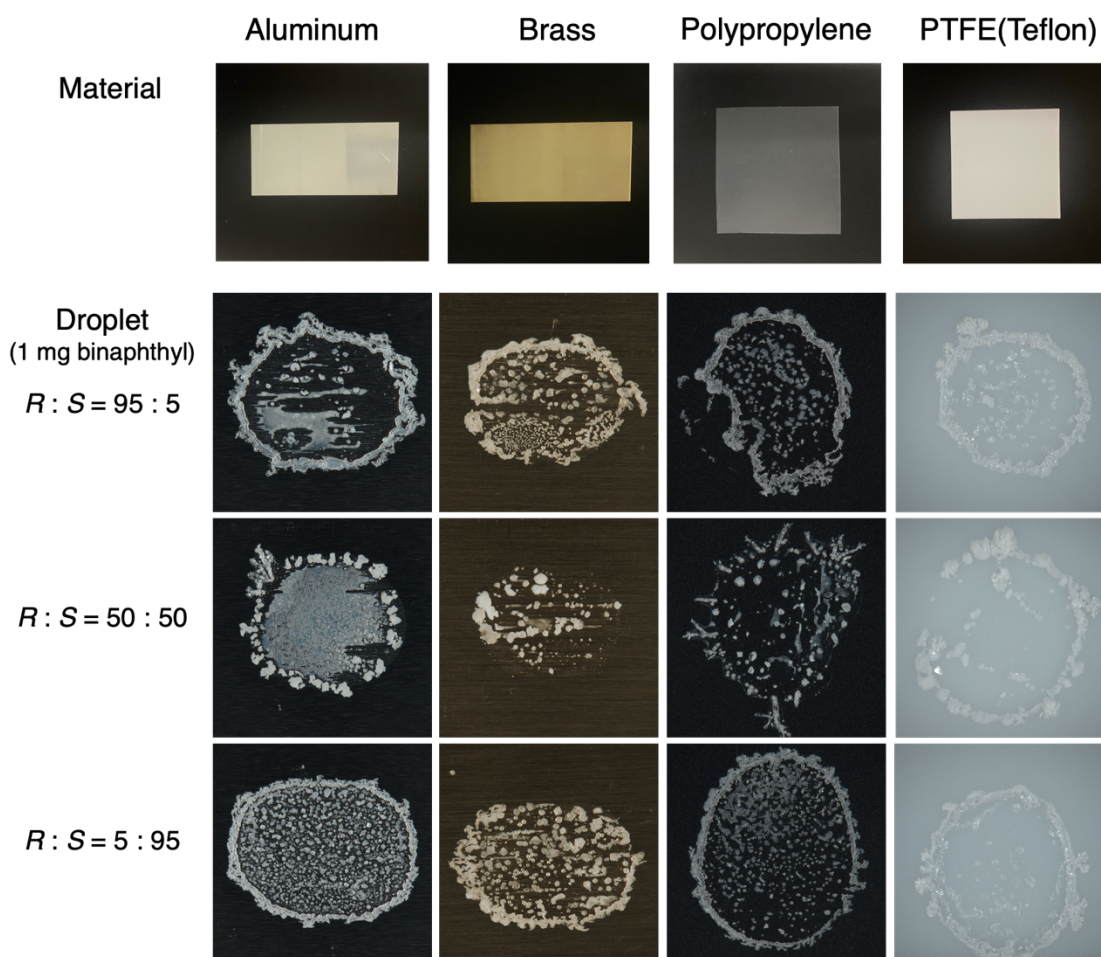


Figure 4-12. Digital-microscope images of 1,1'-binaphthyl droplet after natural evaporation on several materials.

Mixed samples were prepared by mixing (*R*)- and (*S*)-1,1'-binaphthyl solids in arbitrary proportions. Based on the optical purity determined by HPLC analysis, mixing ratio of (*R*)- and (*S*)-1,1'-binaphthyl ($5\% \leq R \leq 95\%$) was calculated and used as training of test data. Mixture was then completely dissolved in benzene at room temperature to create a 50 mg/mL solution. 20 μ L of the solution (equivalent to 1,1'-binaphthyl ca. 1 mg) was gently dropped immediately onto a plate by micropipette, and the solvent was removed by natural drying (**Figure 4-13**). The racemization half-life of 1,1'-binaphthyl in benzene is about 10 hours at room temperature,²¹ change in enantiomeric ratio associated with this operation is negligible. 1,1'-binaphthyl solids after drying were photographed using a previously reported method and linked to the mixing ratio to use as training / test data. For each material, ML model was created from 100 images (training data). Each model was evaluated by 100 images (test data) taken with the same material as the training data to verify the prediction accuracy.

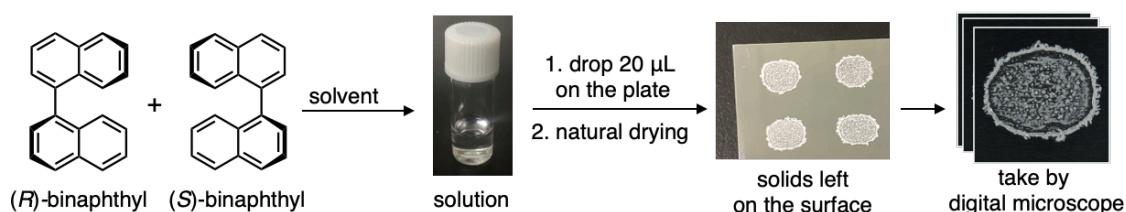


Figure 4-13. Schematic diagram of image collection method.

Aluminum plate showed the highest accuracy among the four surface materials, with an average prediction error of 8.8% and a coefficient of determination of $R^2 = 0.80$ (**Figure 4-14**). This accuracy exceeded that of the model constructed using 550 images obtained from a glass plate (see Figure 4-10), indicating that the surface material can significantly affect the prediction accuracy.

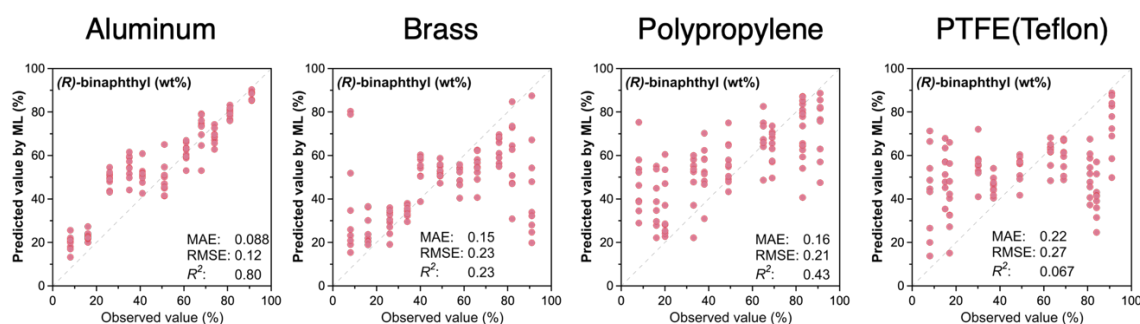


Figure 4-14. Observed-predicted plots for 100 test datasets for (*R*)-1,1'-binaphthyl weight percent under different surfaces (training data: 100 images).

I optimized amount of training data under aluminum plate condition. When the number of training dataset was increased, prediction accuracy improved monotonously. ML model constructed by 330 images of training data showed high prediction accuracy with MAE = 6.8 %, RMSE = 9.2%, and $R^2 = 0.87$ (Figure 4-15a,c). MAE values for 440 and 550 images of training data were 8.6% and 9.5%, respectively, and prediction accuracy did not improve compared to the model with 330 images of training data (Figure 4-15b).

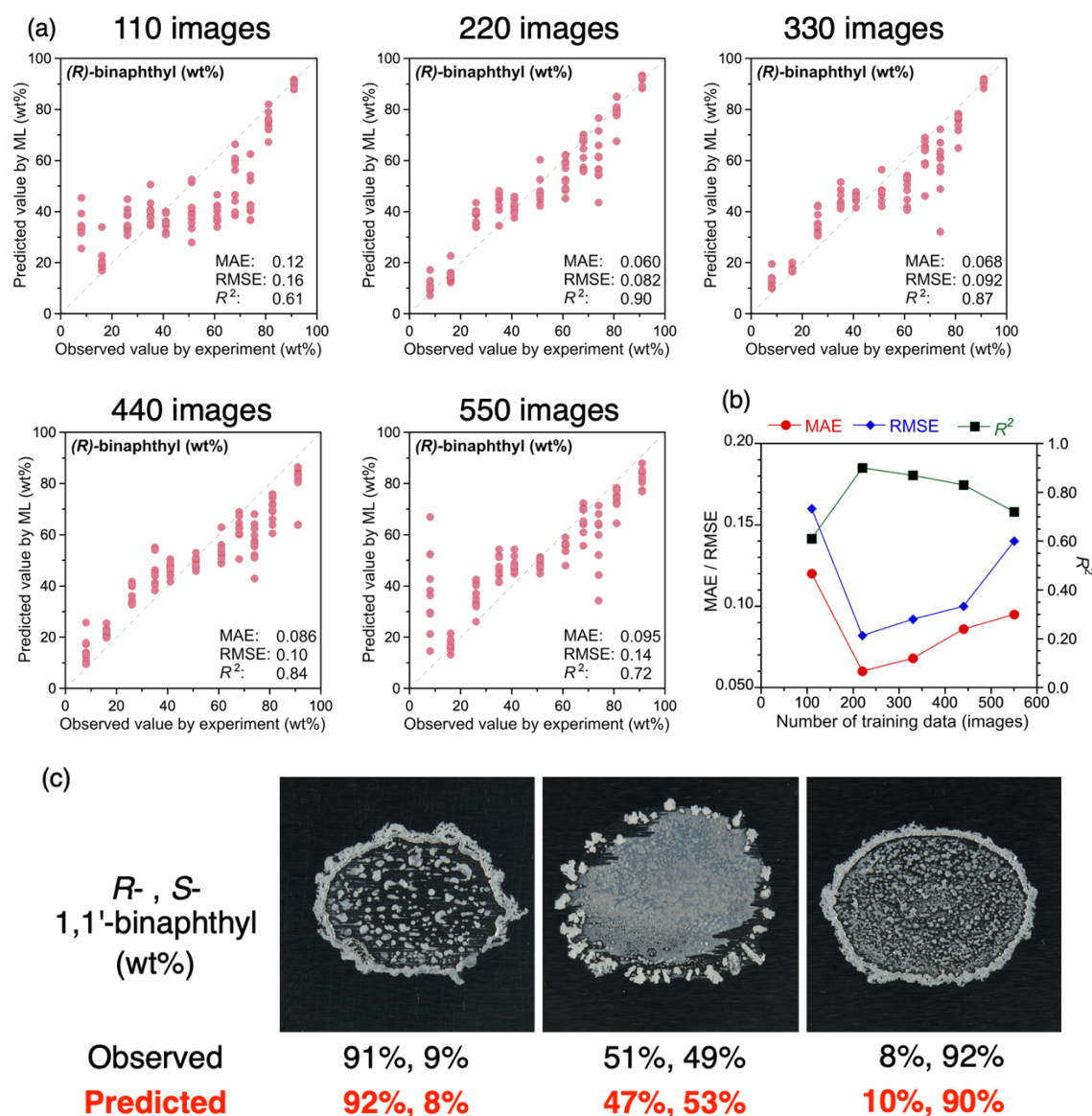


Figure 4-15. (a) Observed–predicted plots for 110 test datasets using different numbers of training images. (b) Relationship between the training image data and the prediction accuracy. (c) Prediction examples for (*R*)-1,1'-binaphthyl weight percent (training data: 330 images).

However, when images taken on different days using the same method were analyzed by ML model, all samples were predicted as racemic (regardless of the true enantiomeric ratio), resulting in a significant decrease in prediction accuracy (average prediction error of 29 %, **Figure 4-16**).

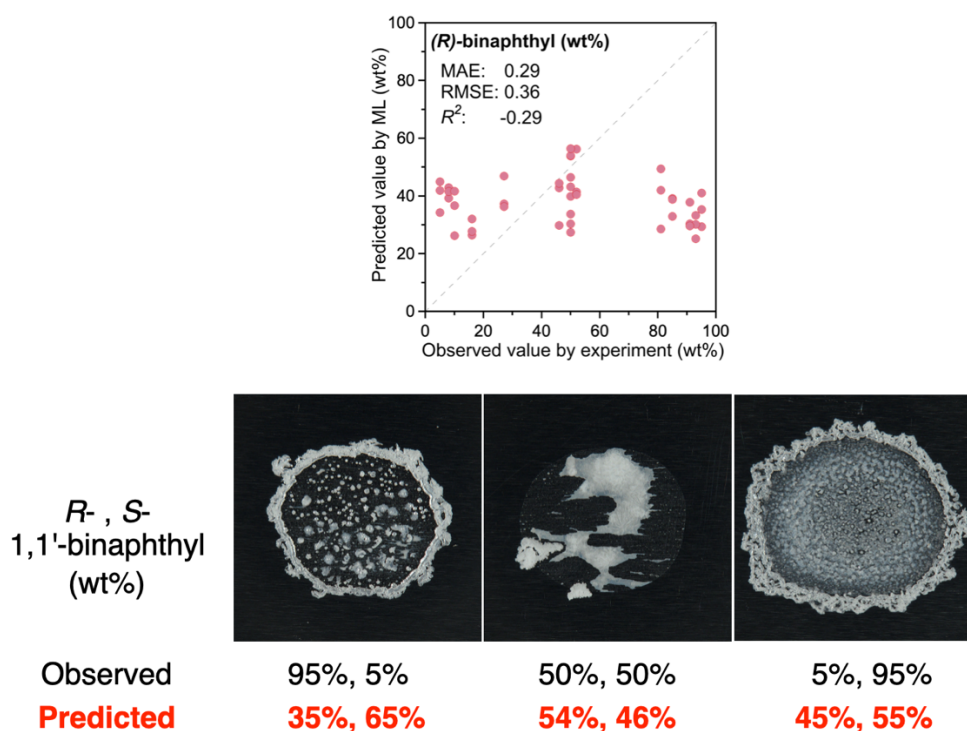


Figure 4-16. Observed-predicted plots for 45 test datasets for (*R*)-1,1'-binaphthyl and prediction examples (training data: 330 images).

The reason for the lower prediction accuracy was the lack of reproducibility due to inevitable differences in the way droplets spread and crystallization behavior under conditions where the solution is dropped on a solid surface. I decided to try another evaporation method to improve practicality.

4.4. Predict Enantiomeric Ratio from Crystalline Solids

As mentioned at Section 4.1., 1,1'-binaphthyl recrystallized from acetone has different crystal structures and solid shapes depending on its optical purity. Therefore, I tried to predict enantiomeric ratio from about 1 mg of crystalline solid obtained by evaporating acetone solution.

Solid 1,1'-binaphthyl sample was prepared for ML. Mixed samples were prepared by mixing (*R*)- and (*S*)-1,1'-binaphthyl solids in arbitrary proportions. 25 mg of 1,1'-binaphthyl was placed in a 10 mL eggplant flask, and 3 mL of acetone was added to completely dissolve it (**Figure 4-17**). After dissolved them, solvent was removed by rotary evaporator. (temperature : 25°C, evaporator pressure : 200 torr, rotation speed : 200 rpm) After complete evaporation, relatively large crystals (0.2-0.8 mm long axis) attached to inside of the flask were collected with a spatula (touched gently to avoid crushing the crystals).

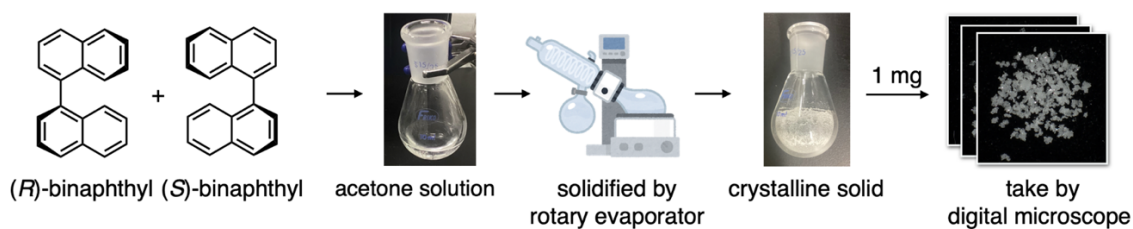


Figure 4-17. Schematic diagram of image collection method.

About 1 mg of crystalline solid was put on a glass plate and photographed with a digital microscope (**Figure 4-18**, magnification: $\times 50$, black background). According to previous studies, binaphthyl chirality half-life in acetone at room temperature is about 12 hours.¹⁵ It means change in the enantiomeric ratio of sample during this procedure is considered negligible. (See **Figure 4-32** in Section 4.9.)

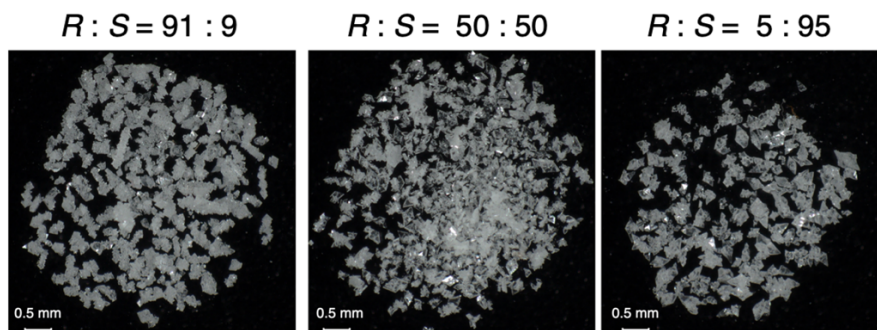


Figure 4-18. Digital microscope images of 1,1'-binaphthyl solids obtained from acetone.

While no correlation was observed between observed and predicted enantiomeric ratios for less than 220 images of training data, strong positive correlation was observed for more than 330 images. Prediction accuracy was almost saturated with 1100 images of training data (MAE = 6.8 %, RMSE = 9.7%, and $R^2 = 0.87$, **Figure 4-19**).

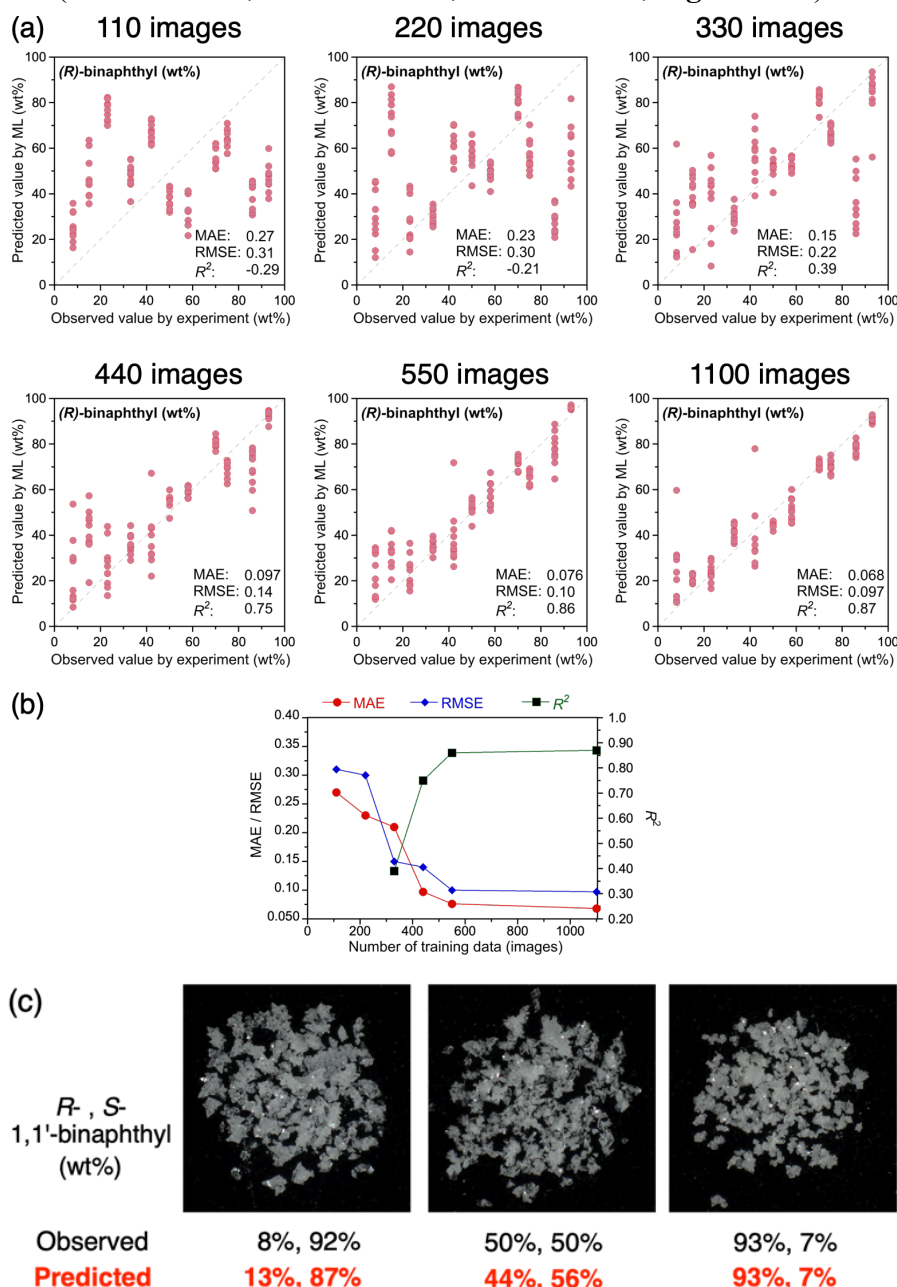


Figure 4-19. (a) Observed–predicted plots for 110 test datasets using different numbers of training images (training data: 1% increment). (b) Relationship between the training image data and the prediction accuracy. (c) Prediction examples for (*R*)-1,1'-binaphthyl weight percent (training data: 1100 images).

As practical prediction in chemical research, chirality of 1,1'-binaphthyl recrystallized from 150°C supercooled melt was predicted by image-based ML (For detailed method to prepare sample, see **Figure 4-31** in Section 4.9.). I prepared 10 samples of 1,1'-binaphthyl from independent symmetry breaking crystallization. Each sample was solidified from acetone solution and photographed by same method as training data. ML model used the optimal conditions shown in **Figure 4-19** (training data: 1100 images). (*R*)-binaphthyl weight ratios predicted from images (averaged for 5 images) and analytical enantiomeric ratios (analyzed by chiral HPLC) are shown in **Figure 4-20**. ML model correctly predict chirality for 6 sample among 10 samples. While prediction for racemic crystal is highly accurate, some samples with high optical purity were predicted by opposite chirality.

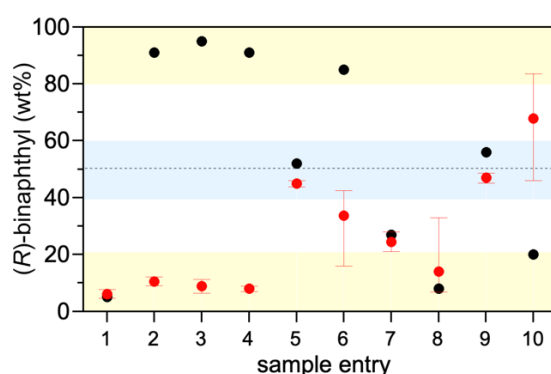


Figure 4-20. Prediction results of (*R*)-binaphthyl ratio (wt%) for 10 samples. (Black : analytical values analyzed by HPLC, red : average predicted value and predicted range (whisker) for each 5 images.)

I focused on absolute value of predicted enantiomeric ratio, in other words, compare enantiomeric excess (*e.e.*). Enantiomeric excess was predicted with mean absolute error of 10.6% (**Figure 4-21**). This result indicates that quantitative distinction between samples with high optical purity and the racemic mixture was achieved by ML model, although there is room for improvement to distinguish *R*- and *S*- isomers.

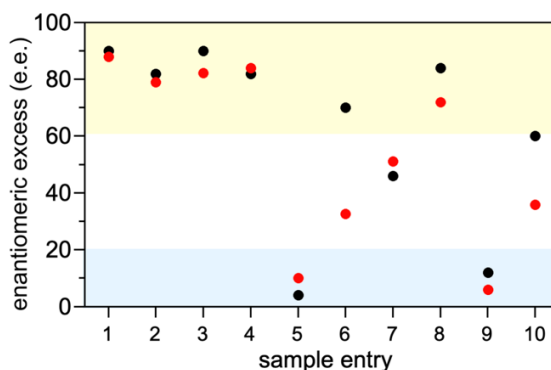


Figure 4-21. Prediction results of enantiomeric excess (*e.e.*) for 10 samples. (Black : analytical values analyzed by HPLC, red : average predicted value.)

4.5. Considerations for Chirality Prediction and Crystal Structures

In order to understand relationship between crystal structure and ML prediction for 1,1'-binaphthyl, sample used for ML was identified by PXRD. PXRD measurements was conducted by sample used in Figure 4-20 and 4-21, solid obtained from 150°C supercooled state and dried from acetone solution. As shown in **Figure 4-22a**, sample used for ML prediction are crystalline solid that exhibits peak pattern in the PXRD measurement, and samples with high optical purity and the racemic mixture can be distinguished from PXRD patterns. Observed PXRD patterns are in good agreement with conglomerate and racemic single crystals reported in the literature.⁴

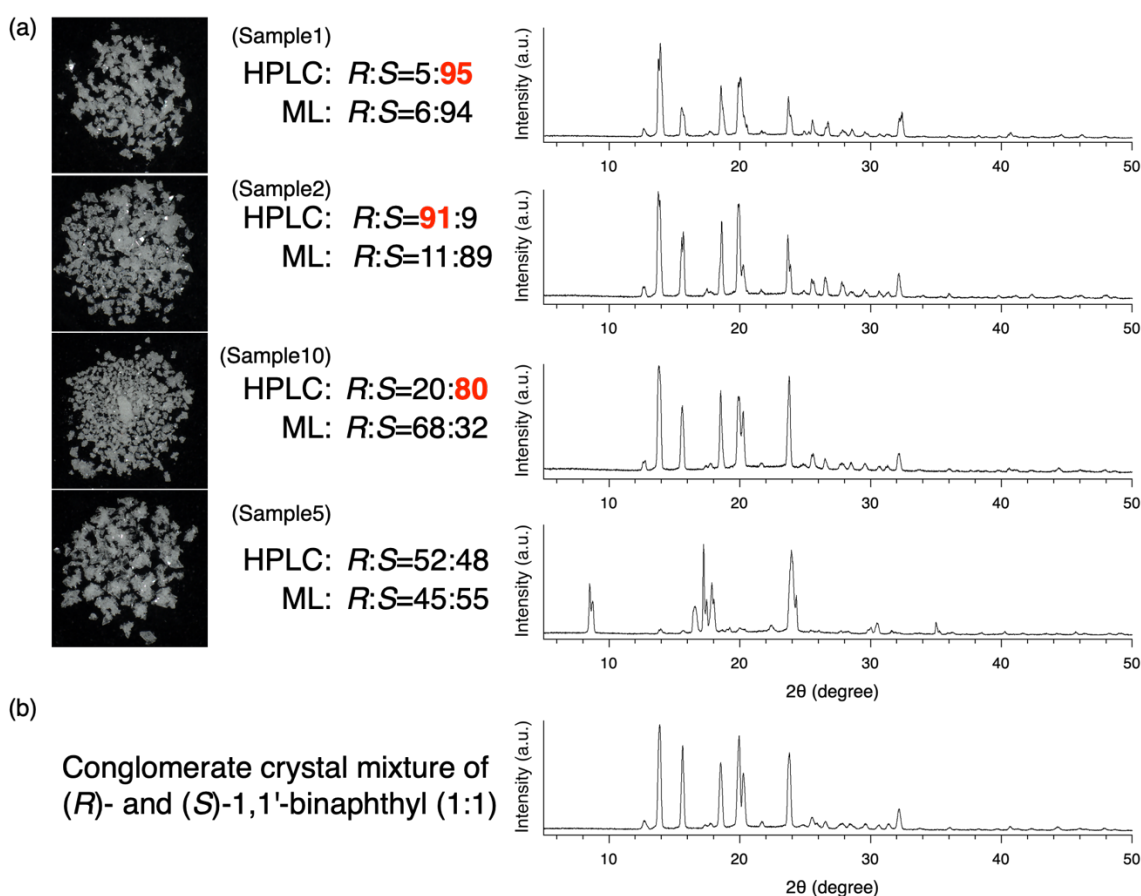


Figure 4-22. (a) 1,1'-binaphthyl solidified from acetone solution. Sample images used for ML prediction, analyzed/prediction of enantiomeric ratios by HPLC/ML, and their PXRD patterns. (for Figure 4-20 and 4-21). (b) PXRD pattern for conglomerate crystal mixture of (*R*)- and (*S*)-1,1'-binaphthyl by 1: 1 weight ratio.

For 90% or higher enantiomeric excess, only chiral crystal patterns were observed. ((*R*)- and (*S*)-1,1'-binaphthyl were obtained as separate crystal). For 5% or less enantiomeric excess, only racemic crystal patterns were observed ((*R*)- and (*S*)-1,1'-binaphthyl form co-crystal). For moderate enantiomeric excess, racemic crystal peak was slightly observed in chiral crystal pattern.

On the other hand, PXRD patterns were almost identical for (*R*)- and (*S*)-1,1'-binaphthyl, difficult to distinguish chirality from PXRD analysis. For comparison, conglomerate crystals mixture of (*R*)- and (*S*)-1,1'-binaphthyl were grinded and mixed at a 1: 1 weight ratio were measured (**Figure 4-22b**). Although this sample is racemic mixture, PXRD pattern was coincidence with conglomerate crystals of (*R*)- and (*S*)-1,1'-binaphthyl. (*R*)- and (*S*)-1,1'-binaphthyl could not be distinguished by PXRD analysis.

These results suggest that ML model may be predict enantiomeric excess of 1,1'-binaphthyl based on visual information derived from crystal structures. Even for chemically identical racemic mixtures, different PXRD patterns were detected depending on sample preparation method. This suggests that it is important to predict optical purity using crystalline solids obtained from homogeneous solvent, rather than simply mixing solids obtained from supercooled state. I thought that this ML system could be used as a preliminary screening to select samples with high optical purity from a large number of experimental conditions that may bias chirality.

4.6. Predict Enantiomeric Ratio via Polarization Light

I tried to use polarized light for ML prediction, in order to obtain visual information that is difficult to obtain by a digital microscope. Solids used this experiment showed almost identical crystal shapes, would be difficult to distinguish based on shape information alone. When solids were observed through a polarizing plate, polarization characteristics was observed. Color and transparency changed depending on the orientation of the polarizing plate. To reflect polarization characteristics of the chiral crystal as image information, I photographed samples with a polarizing microscope.

1,1'-binaphthyl sample was obtained by same method mentioned at Section 4.5. (Obtained from acetone solution, see Figure 4-17.). Approximately 1 mg of the solid after drying was arranged on a glass plate, and images were taken using a polarizing microscope with two polarizing plates fixed at an angle of 45° (**Figure 4-23**).

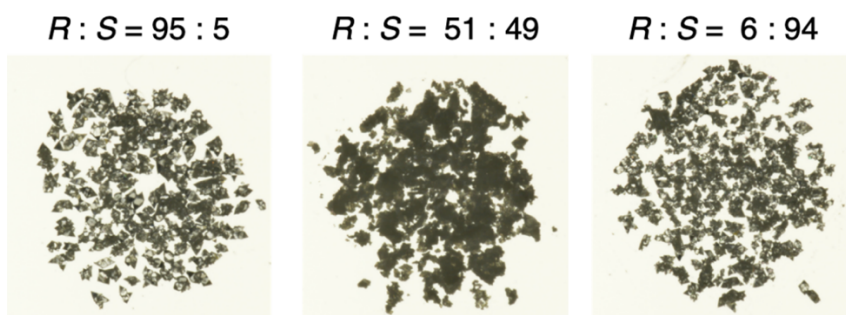


Figure 4-23. Polarized-microscope images of 1,1'-binaphthyl solids after evaporation from acetone.

The model constructed from 550 images as training data can distinguish between the (*R*)- and (*S*)-1,1'-binaphthyl with prediction error of MAE = 14%, RMSE = 19%, and coefficient of determination $R^2 = 0.47$ (**Figure 4-24**).

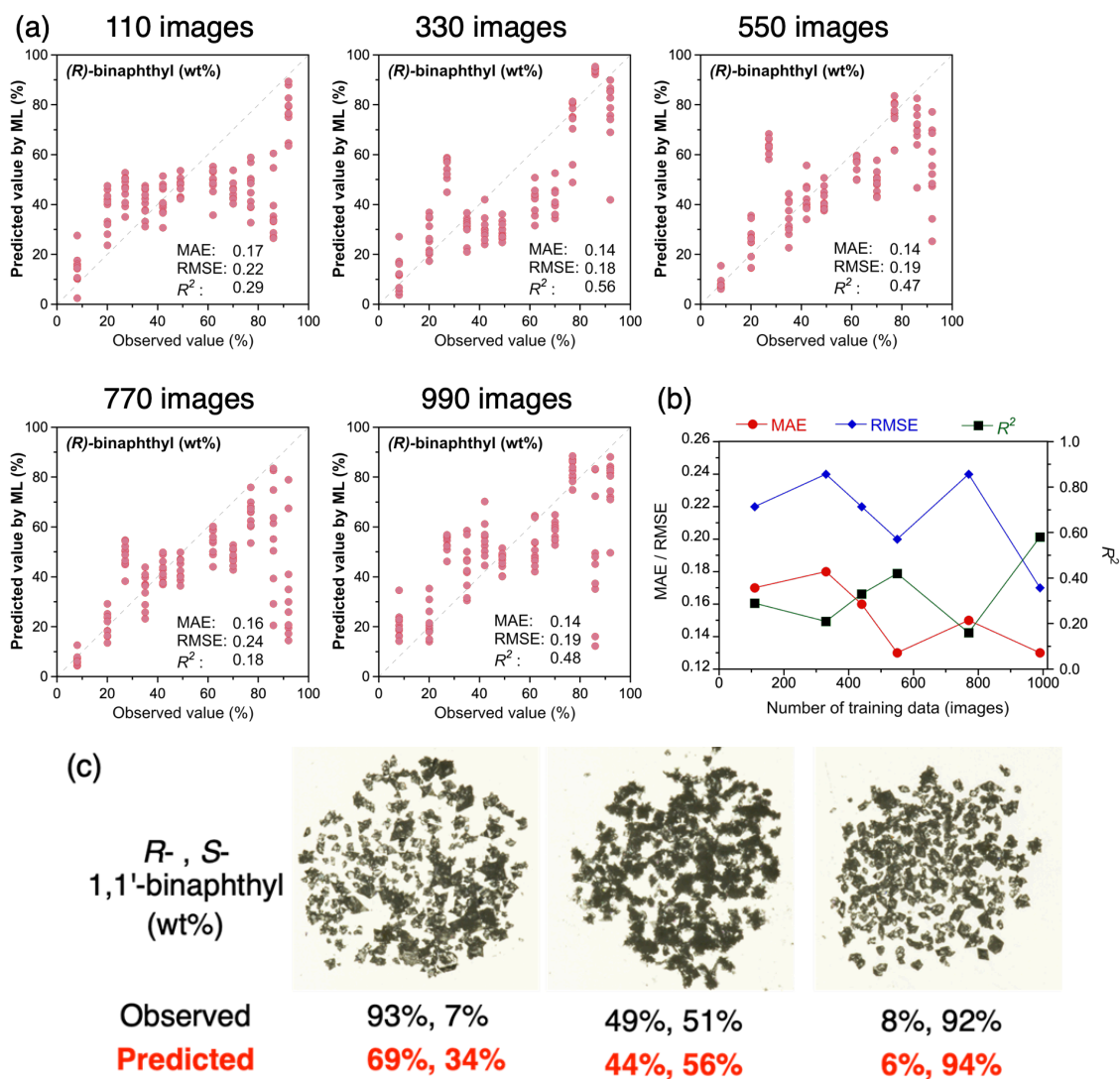


Figure 4-24. (a) Observed–predicted plots for 110 test datasets using different numbers of training images. (b) Relationship between the training image data and the prediction accuracy. (c) Prediction examples for (*R*)-1,1'-binaphthyl weight percent (training data: 550 images).

20 samples of 1,1'-binaphthyl were prepared to confirm that ML model could be used obtained from recrystallized supercooled melt. As **Figure 4-25**, 10~12 samples were correctly predicted as (*R*)- or (*S*)- 1,1'-binaphthyl. No significant improvement in accuracy was observed to change brightness of polarized light, ML settings, and solidification methods. Although total prediction accuracy of was 60-70%, there may be some relationship between chirality and appearance of the crystals, because predictions are correct for more than half of all samples (See Section 4.5.).

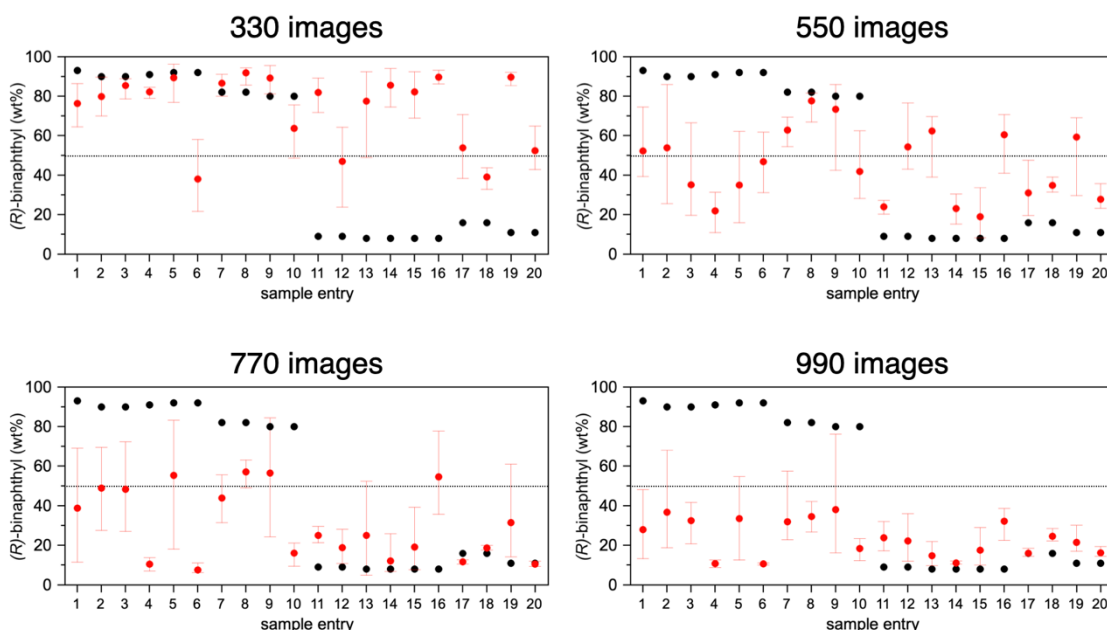


Figure 4-25. Prediction results of (*R*)-binaphthyl ratio (wt%) for 20 samples with different amount of training dataset. Black : analytical values analyzed by HPLC, red : average predicted value, and predicted range (whisker) for each 10 images.

To confirm predictability of samples recrystallized from supercooled state, attempted cross-validation prediction. 100 samples of chiral sample recrystallized from supercooled state (500 images, 5 images for each 1 sample) were divided into five groups (A, B, C, D, E), each containing 20 samples (100 images). For each prediction, one group was used as test data, and remaining four groups (400 images) and racemic mixture (20 samples, 50 images) were used as training data. Prediction accuracy was over 60% for all combinations of training/test data (**Figure 4-26**).

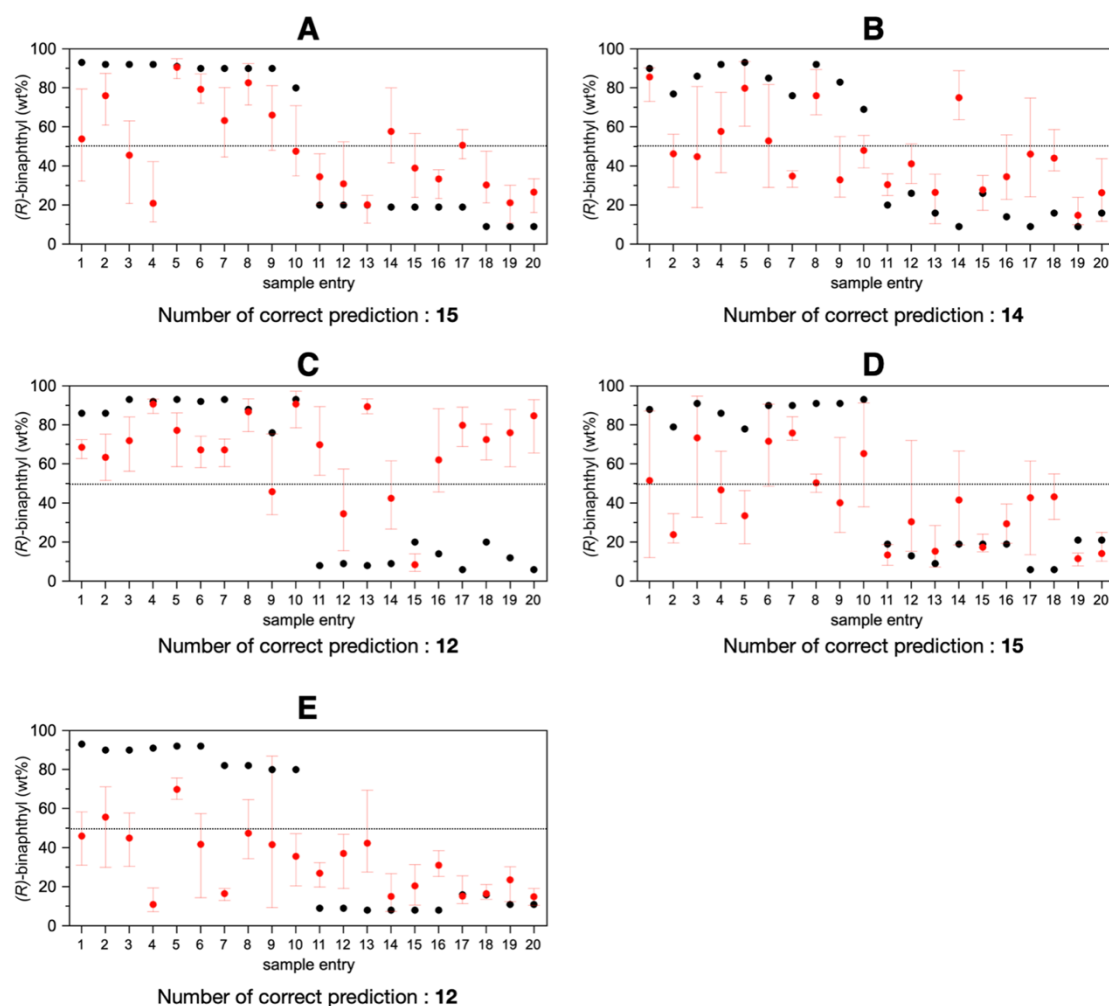


Figure 4-26. Prediction results of (*R*)-binaphthyl ratio (wt%) for 20 samples with different combination of training/test dataset. (Black : analytical values analyzed by HPLC, red : average predicted value, and predicted range (whisker) for each 10 images.) Training data : 450 images (100 samples), test data : 200 images (20 samples).

The prediction accuracy of chirality did not improve dramatically with the use of polarizing light. However, prediction accuracy was above 50% for every combination of training and test data, suggesting that there may be some relationship between chirality and crystal appearance.

4.7. Improve Detection Limit of Isopyrazole Metal Ion Sensor

Image-based ML was applied to improve the performance of the metal ion sensor **1** shown in **Figure 4-27** (Detailed synthesis method is shown in Chapter 2).¹⁶

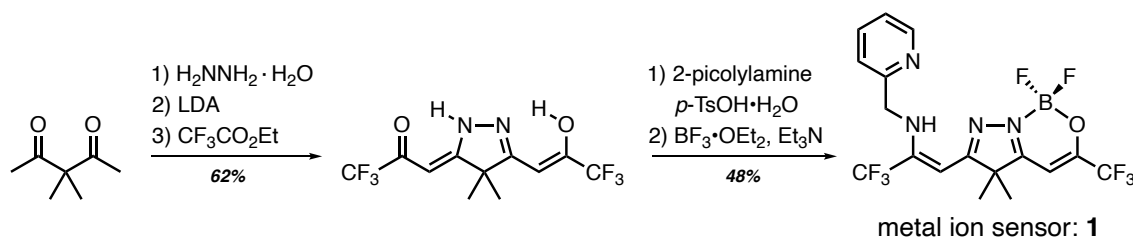


Figure 4-27. Synthesis scheme of metal ion sensor compound **1**.

I tried quantitative prediction of metal ion difficult to distinguish by naked eye. Aiming to detect Zn^{2+} ion like pH-test paper, ML data was collected by soaking filtration paper with a compound **1** solution containing Zn^{2+} ions at an arbitrary stoichiometric ratio. To a solution of compound **1** (21 mM, solvent: $\text{CH}_2\text{Cl}_2/\text{MeOH}=1:1$, v/v), $\text{Zn}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ was added by arbitrary condensation (0~31.5 mM), and one drop of (5 μL) solution was dripped onto a filter paper. After dried sufficiently, filtration paper (contain approximately 48 μg of compound **1** and 0~5.2 μg of Zn^{2+} ion) was photographed with a digital microscope. Sample images showed slight changes in appearance depending on the equivalent of Zn^{2+} ion, but difficult to quantify them with the naked eye (**Figure 4-28**).

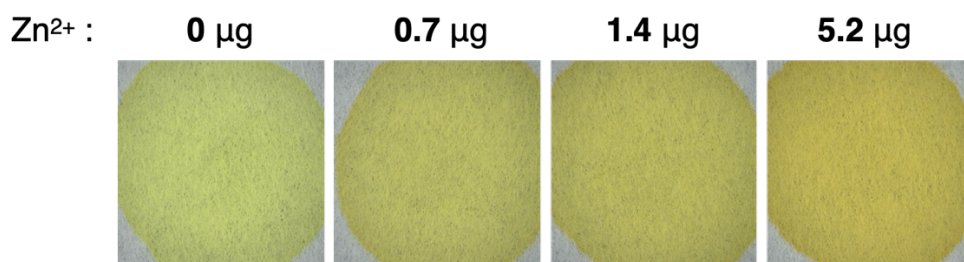


Figure 4-28. Digital-microscope images of compound **1** with Zn^{2+} ion droplets naturally evaporated on filter paper.

ML model constructed by 160 training images was found to correctly predict amount of Zn^{2+} ion for more than $0.35 \mu\text{g}$ Zn^{2+} ion (**Figure 4-29**). It means that image-based ML can improving quantitative performance for compound **1** by improving identification performance.

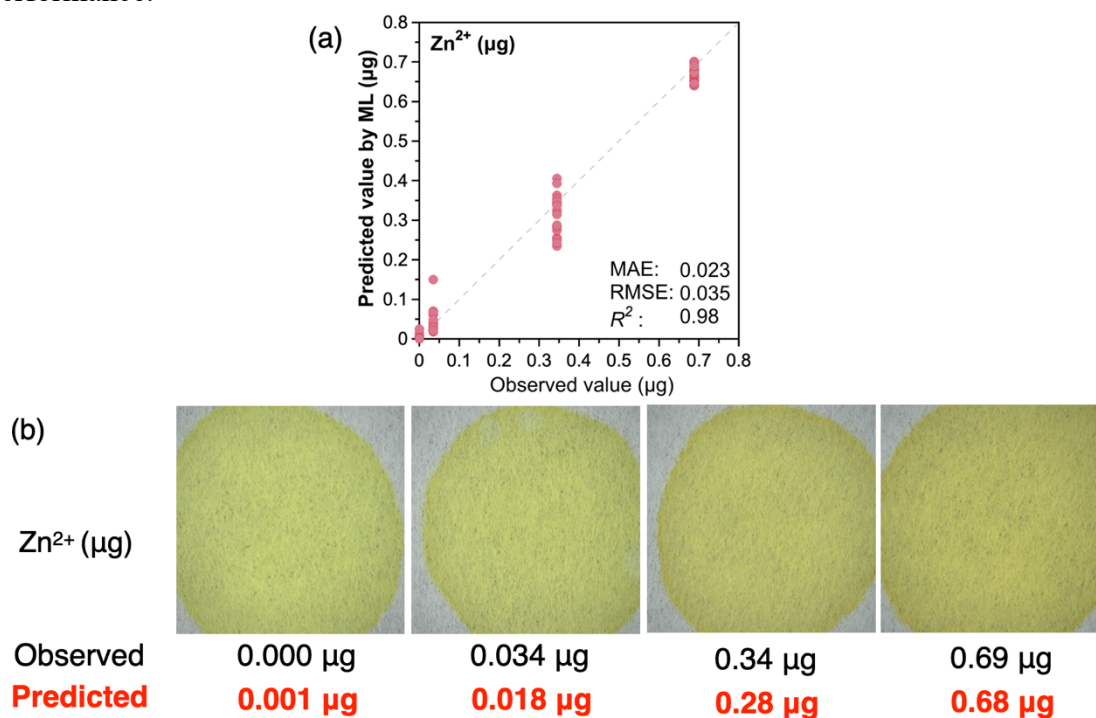


Figure 4-29. (a) Observed–predicted plots for 80 test datasets (training data: 160 images). (b) Prediction examples for amount of Zn^{2+} ion weight percent.

This result shows that improvement accuracy of molecular sensors was achieved by image-based ML, which was the starting point of this research. Even subtle changes that are difficult to detect with naked eye can be used to improve performance of ion sensors to a practical level by image-based ML.

4.8. Conclusion

I attempted to apply ML method to the rapid determination of the enantiomeric ratio of randomly biased (*R*)- or (*S*)-1,1'-binaphthyl obtained by symmetry breaking techniques, and improve performance of isopyrazole molecular sensor.

I tackled prediction of (*R*)- or (*S*)-1,1'-binaphthyl in two ways, benzene solution dried dropwise, and acetone solution drying under reduced pressure. However, neither method had sufficient prediction accuracy for samples prepared on a different day from training data. Both methods were successful in predicting the enantiomeric ratio of the same samples with an accuracy of within 10%. The prediction of enantiomeric excess obtained from supercooled state was also successful. Although prediction accuracy between (*R*)- or (*S*)-1,1'-binaphthyl was 60-70%, enantiomeric excess was successfully predicted with an average error of approximately 10%, in other words, possible to distinguish between chiral crystals and racemic crystals. Because ML is not omnipotent, I thought that 1,1'-binaphthyl would be difficult to predict from the image-based ML. I thought that application possibility of ML prediction can remain by easier to distinguish differences in shape of (*R*)- or (*S*)-1,1'-binaphthyl by controlling solid state shape in chiral environment. Crystal shape of organic chiral compounds can be controlled to change crystal growth rate by trace amounts of chiral additives.^{22,23}

For fluorescent isopyrazole metal ion sensor **1**, succeeded to quantitative prediction for Zn^{2+} by image-based ML. Detection performance of metal ion sensor was improved than naked-eye identification by solid-state fluorescence, demonstrated that ML systems can be applied to improve the performance of molecular sensor. This methodology may also be useful for improving performance of other molecular sensors by improving distinction capabilities. These application examples suggest that image-based ML has the potential to make further contributions to chemical research.

4.9. Experimental Section

General Information

Solvents and reagents were purchased from FUJIFILM WAKO Pure Chemical Industries Ltd., TCI Co., Ltd., and Sigma-Aldrich Co., and used without further purification. 1,1'-binaphthyl was purchased from TCI Co., Ltd. as racemic mixture, and prepared as (*R*)- and (*S*)-1,1'-binaphthyl by reported procedures described below. Other solvents and reagents were purchased from WAKO Pure Chemical Industries Ltd., Kanto Chemical Co., Inc., and TCI Co., Ltd. and used without further purification unless otherwise mentioned. Glass plate and metal plates was purchased by Matsunami Glass Ind., Ltd. or Taiho Trading Co. Ltd. and used without further operations. Analytical HPLC chromatograms were recorded using a JASCO MD-4010 photodiode array detector equipped with a JASCO PU-4580 pump, JASCO AS-4550 autosampler, and JASCO CO-4065 column oven. Filter paper was purchased by ADVANTEC (filter paper No. 1) and used without further purification. Powder X-ray diffraction (PXRD) spectra were measured with MiniFlex600-C X-ray diffractometer (CuK α radiation λ = 1.54059 Å). Synthesis of isopyrazole compound **1** followed methods described in Chapter 2. ML settings followed method described in Chapter 3 unless otherwise noted.

HPLC analysis of 1,1'-binaphthyl

Enantiomeric ratio of 1,1'-binaphthyl was analyzed by a chiral HPLC based on reported procedure.²⁴ 0.50 mg of 1,1'-binaphthyl was completely dissolved in 1.0 mL mixed solvent (hexane : isopropyl alcohol = 99 : 1 (v/v)). Sample solution was analyzed by CHIRALPAK® IB N-3 (temperature: 20°C, flow rate: 1.0 mL/min). Example of the analysis results is shown in **Figure 4-30**. Optical rotation measurements confirmed that the peak of (*R*)-1,1'-binaphthyl was observed at shorter retention time than that of (*S*)-1,1'-binaphthyl.

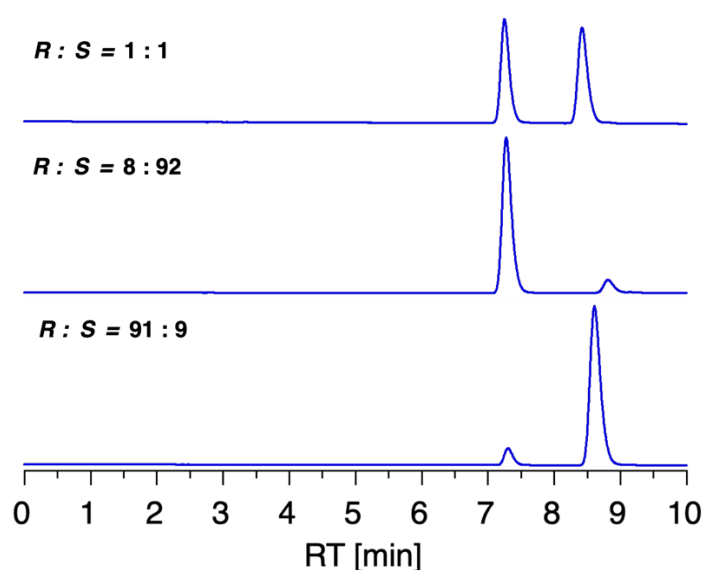


Figure 4-30. HPLC analysis of racemic mixture and (*R*)- and (*S*)-1,1'-binaphthyl.

Preparation of (*R*)- and (*S*)-1,1'-binaphthyl

(*R*)- and (*S*)-1,1'-binaphthyl were prepared according to the reported procedure.² In a 10 mL round-bottomed flask with magnetic stirrer, 1 g of 1,1'-binaphthyl was melted by heating to 180°C with stirring. After maintained 180°C for about 20 min, gradually cool to 150°C with stirring. After complete crystallized, optical purity was determined using chiral HPLC analysis and used. In many cases, crystals with more than 80% *e.e.* (in other words, $R:S = \geq 90 : 10$ or $R:S = 10 : \geq 90$) were obtained. After repeating the experiment 12 times, (*R*)- and (*S*)-1,1'-binaphthyl were randomly obtained with almost half-and-half probability (**Figure 4-31**) as described in reported literature.

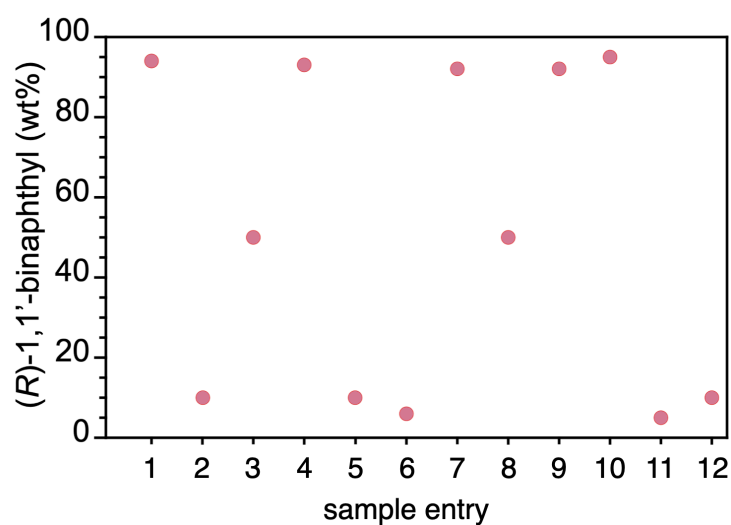


Figure 4-31. Chirality distribution (analyzed by HPLC) of 12 samples crystalized from 150°C supercooling state.

Confirm chirality of 1,1'-binaphthyl obtained by experimental procedure

Although previous studies had indicated that chirality changes in acetone within 1 hours were negligible,²¹ it was also reported that racemization could be enhanced by specific impurities in solution. So, I confirmed whether the method of acquiring 1,1'-binaphthyl sample image data affect the chirality itself.



For the four chiral 1,1'-binaphthyl samples (obtained by method mentioned in **Figure 4-1** and related references),² the chirality of the same samples immediately and after applying the dry solidification method shown in Section 4.4. (shown in **Figure 4-17**) were compared by chiral HPLC (**Figure 4-32**). The observed enantiomeric ratios were within the error range associated with the HPLC analysis and preparative manipulation and considered to be not affect the machine learning results. From this experiment, change in the chirality due to acetone dissolved and solidified can be ignored.

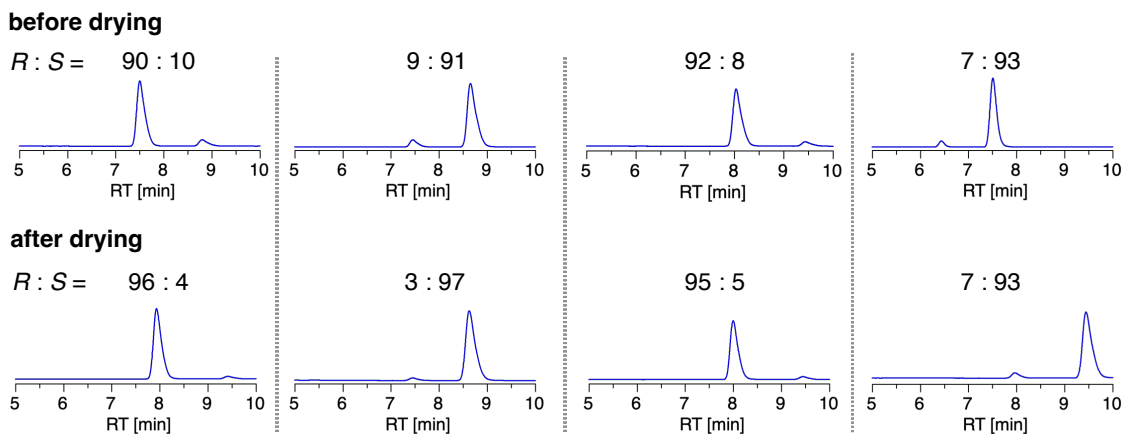


Figure 4-32. HPLC analysis for (*R*)- and (*S*)- 1,1'-binaphthyl before and after drying method.

4.10. References

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Chapter 5

Conclusion and Future Outlook

5.1. Conclusion

In this thesis, the author has demonstrated the applicability of image-based machine learning (ML) in chemical research for predicting mixing ratios such as polymorphism and enantiomeric ratios, chemical reaction yields, and improving the performance of fluorescence ion sensors.

In Chapter 2, showed synthesized BF₂ isopyrazole complex have ability of metal ion sensor. Yellow-green fluorescence of BF₂ complex changed to yellow in the presence of Zn²⁺ ion, while fluorescence was quenched in the presence of Cu²⁺, Ni²⁺, and Co²⁺ ions. Those fluorescence changes by metal ions were observed both in organic solvents and solid state. In solid state, visible changes in fluorescence were observed even for μg of metal ions. However, detection limit of metal for naked eye was about 1 μg, because visual changes are difficult to distinguish. I thought image-based ML might improve detection limits.

In Chapter 3, investigated whether image-based ML could be used to objectively predict for solid compound mixtures. ML system was optimized as a pilot model to predict commercially available sugar and dietary salt. After several optimizations, ML model constructed from 300 images of sugar and dietary salt mixtures can predict these weight ratios with an average error of 3.9%. The accuracy and speed of the predictions exceeds that of humans. Using mobile cameras and stand-alone prediction systems, ML system can be used as location-independent analysis method. α-glycine and γ-glycine, which is crystal polymorphs, can distinct them from the images with an error of 4.0%, in the same way as sugar and dietary salt. Same method can also be applied to the enantiomeric ratio of L-/D-tartaric acid and multi-component mixture. Furthermore, solid chemical reaction yield can successfully be predicted by inputting images of the reaction mixture and ¹H NMR yields with an error of 5.7%. It was possible to successfully predict changes in reaction yield with little effort using videos of the reaction process.

In Chapter 4, apply image-based ML system to chemical research to predict enantiomeric ratio for trace amount of solids and presence of metal ions by isopyrazole metal ion sensor. For 1,1'-binaphthyl, successfully predicted enantiomeric excess with an average error of about 10% by 1 mg solids obtained from solvents. I also succeeded in quantitatively detecting sub-μg metal ion, using isopyrazole metal ion sensor developed in Chapter 2.

These results indicate that image-based ML has potential as a tool to objectively analyze visual information of solid compounds, which have been difficult to use as analytical methods using conventional procedures. This research has the potential to lead to greater efficiency and advancements in a variety of chemical research.

5.2. Future Perspectives

This thesis proposed a concept of the applicability of image-based ML in chemical research, with show some examples for scope and limitations. Recently, our laboratory reported rapid quantification of hydride content in perovskite hydrides ($\text{BaTiO}_{3-x}\text{H}_x$) by image-based ML.¹ Although non-destructive and highly accurate instrumental analysis of perovskite hydride is difficult, ML system can successfully monitor reaction and control hydride content. However, the number of predictive targets analyzed by image-based ML is still small, around 20. To reaching the ultimate goal of "quantifying all chemical reactions using image-based ML", further study is needed on, such as image-taking conditions for highly accurate predictions can be achieved, the tendencies of analysis targets which predictions are difficult.

One of concerns for the practical application of this system is the need to acquire a large number of images as training data. However, this concern could be alleviated by combining with an experimental robot.^{2,3} These experimental robots can automatically assemble a given chemical experiment and plan a new reaction based on the reaction results such as spectral analysis, in a fully automated manner. If procedure for acquiring image data of the reaction mixture could be incorporated into these experimental robots, ML model could be constructed without a great deal of human effort and time.

I also considered that chemical parameters not directly derived from the mixing ratio could also be subject to analysis. For example, the viscosity of a solution is an important parameter for reaction rate and reactivity.^{4,5} On the other hand, humans can predict viscosity sensibly from images and videos, and ML systems have been developed based on such prediction methods.⁶ I thought that by applying this knowledge, it would be possible to objectively estimate viscosity from solvent videos. In this way, it can be said that image-based ML still has further potential for applications.

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List of Publications

Chapter 2

Isopyrazole-Masked Tetraketone: Tautomerism and Functionalization for Fluorescent Metal Ligands

H. Shirakura, Y. Manabe, C. Kasai, Y. Inaba, M. Tsurui, Y. Kitagawa, Y. Hasegawa, T. Yoneda, Y. Ide, Y. Inokuma,
Eur. J. Org. Chem. **2021**, 30, 4345-4349.

Chapter 3

Machine Learning-Based Analysis of Molar and Enantiomeric Ratios and Reaction Yields Using Images of Solid Mixture

Y. Ide, H. Shirakura, T. Sano, M. Murugavel, Y. Inaba, S. Hu, I. Takigawa, Y. Inokuma,
Ind. Eng. Chem. Res. **2023**, 62, 13790-13798.

Other Publications

Modular Synthesis of Oligoacetylacetones via Site-selective Silylation of Acetylacetone Derivatives

P. Sarkar, Y. Inaba, H. Shirakura, T. Yoneda, Y. Inokuma,
Org. Biomol. Chem. **2020**, 18, 3297-3302.

Insoluble π -Conjugated Polyimine as an Organic Adsorbent for Group 10 Metal Ions

H. Shirakura, Y. Hijikata, J. Pirillo, T. Yoneda, Y. Manabe, M. Murugavel, Y. Ide, Y. Inokuma,
Eur. J. Inorg. Chem. **2021**, 17, 1705-1708.

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