Simple demonstration of using the IQA software

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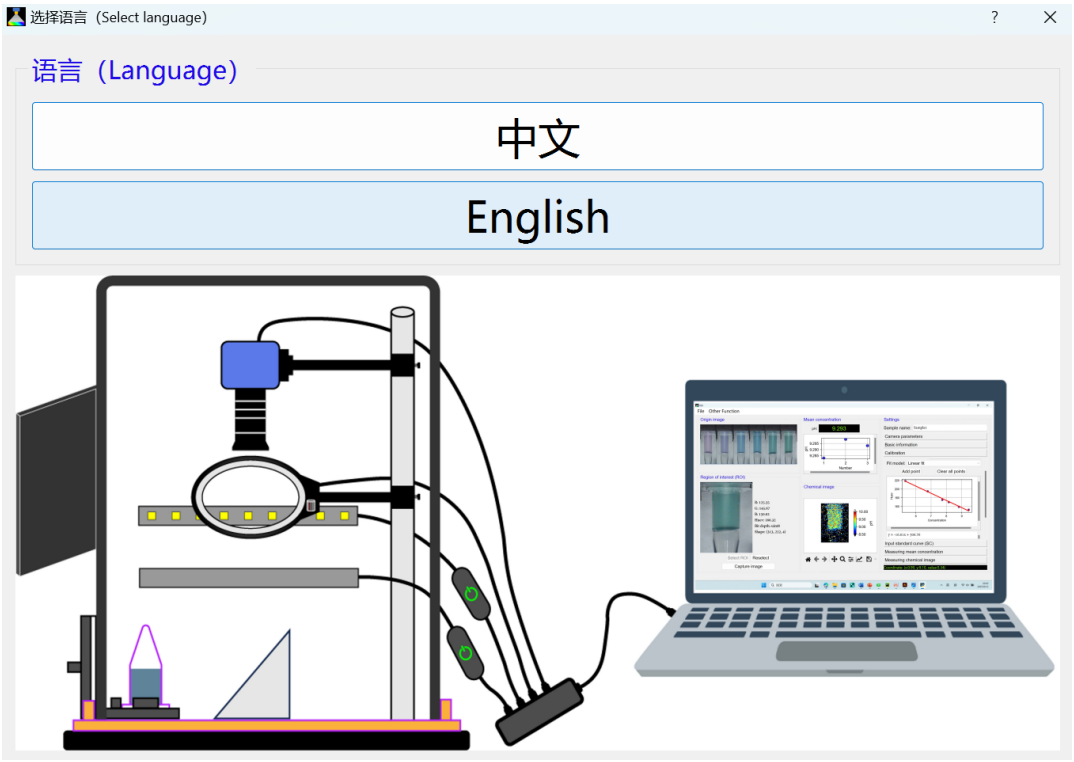
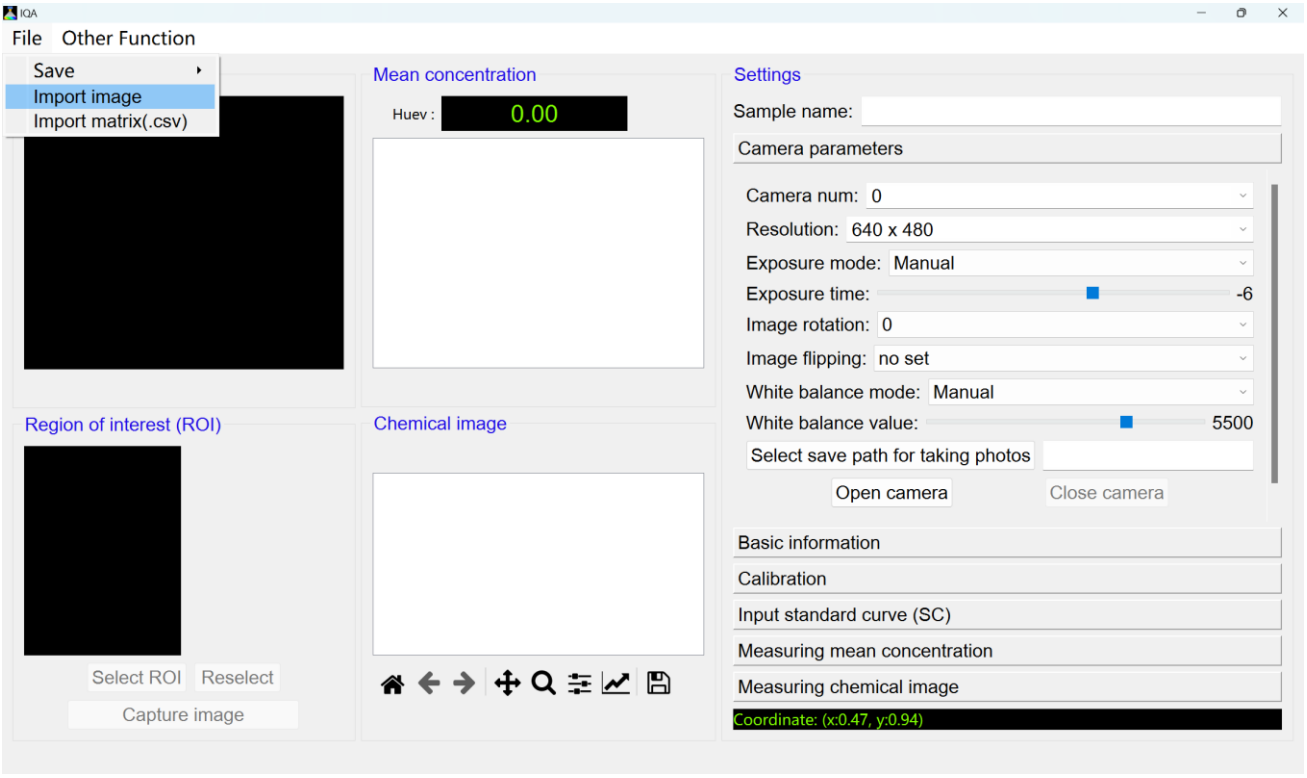
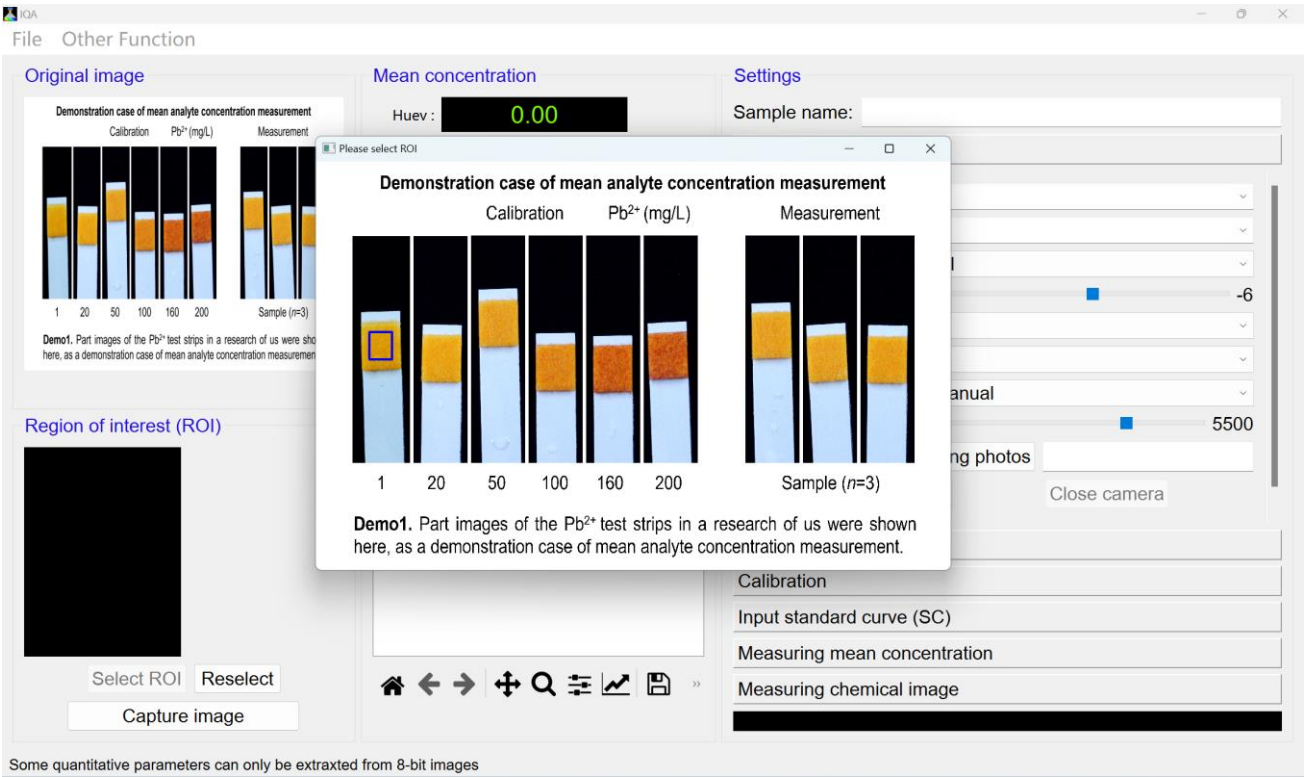
1. Selection of GUI language. Here, we selected English.



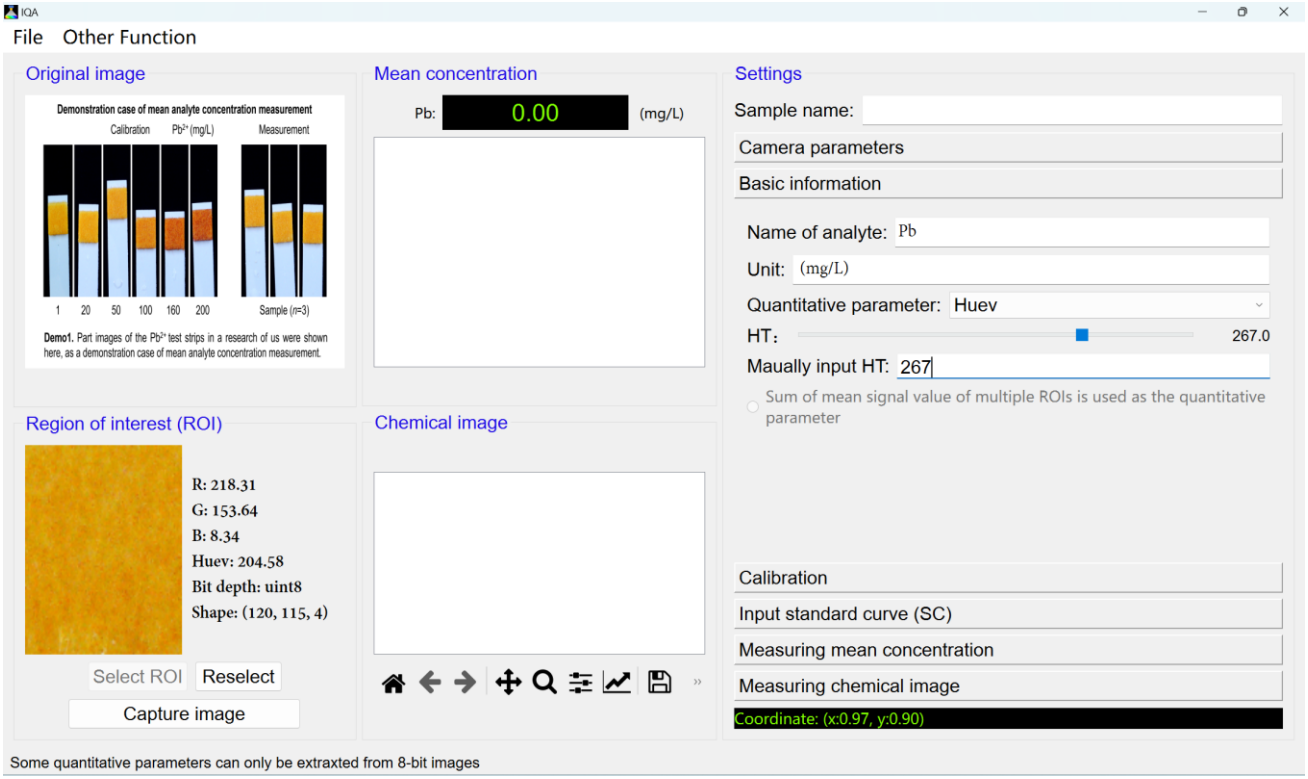
2.Demonstration of mean analyte concentration measurement.

(1) Import the image to be demonstrated and select the Region of Interest (ROI).

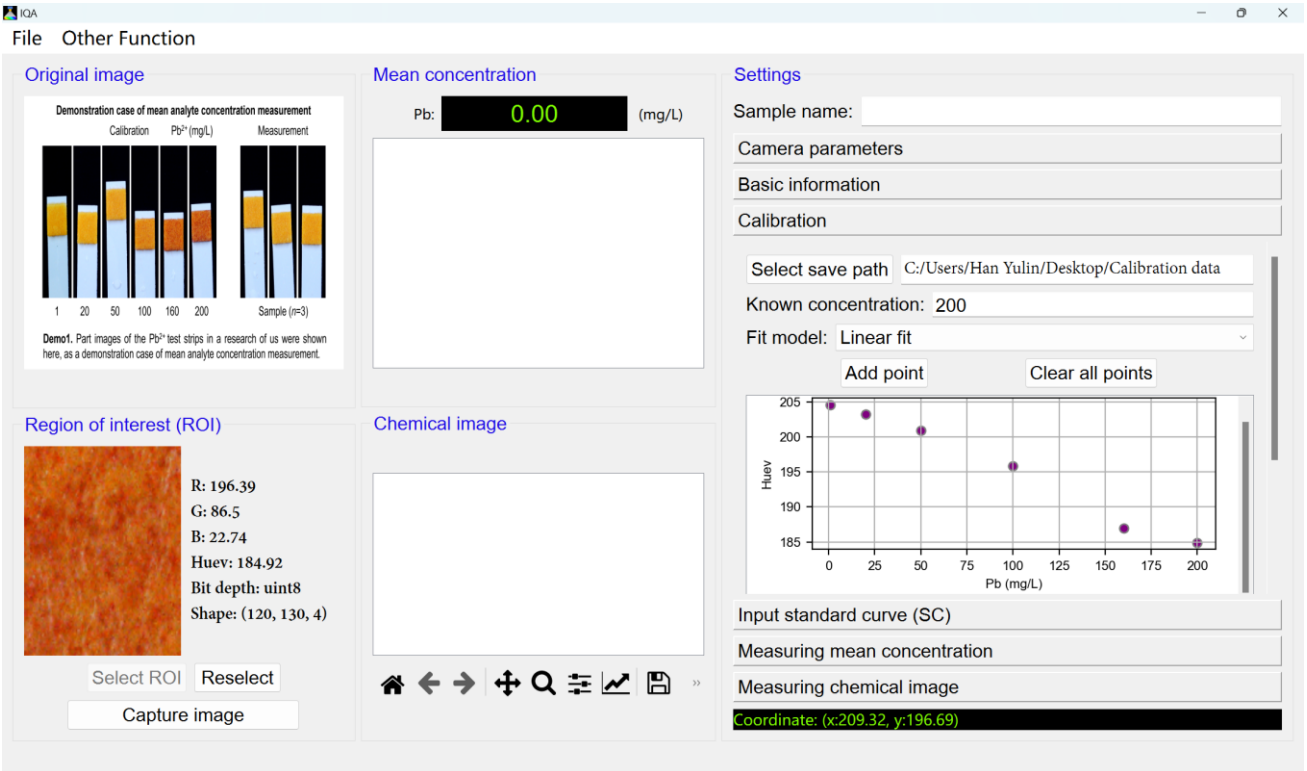




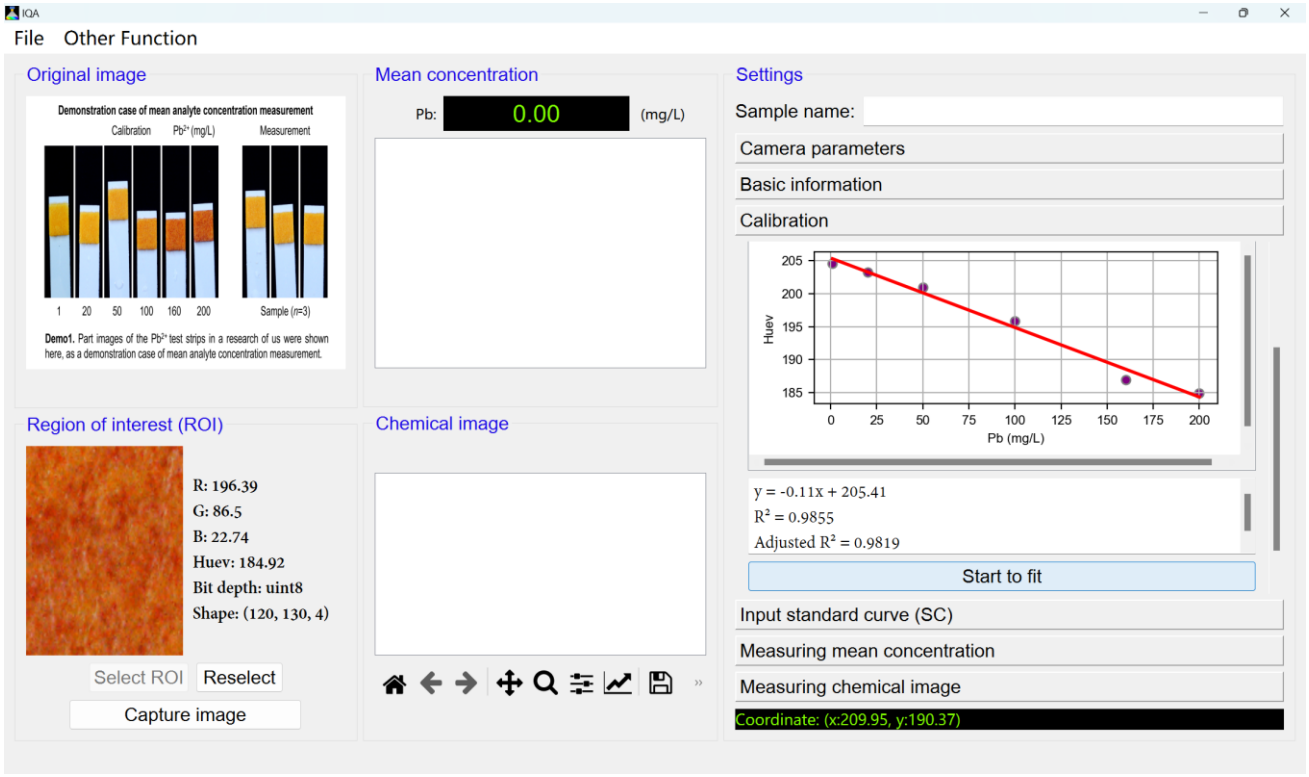
(2) Set basic information. Note that the value of HT needs to be set if Hue_v (presented as Huev in IQA) is used as the quantitative parameter. The method for selecting the HT value was described in a research article of us. The optimal HT for the Pb^{2+} test strips used in that article is 267.

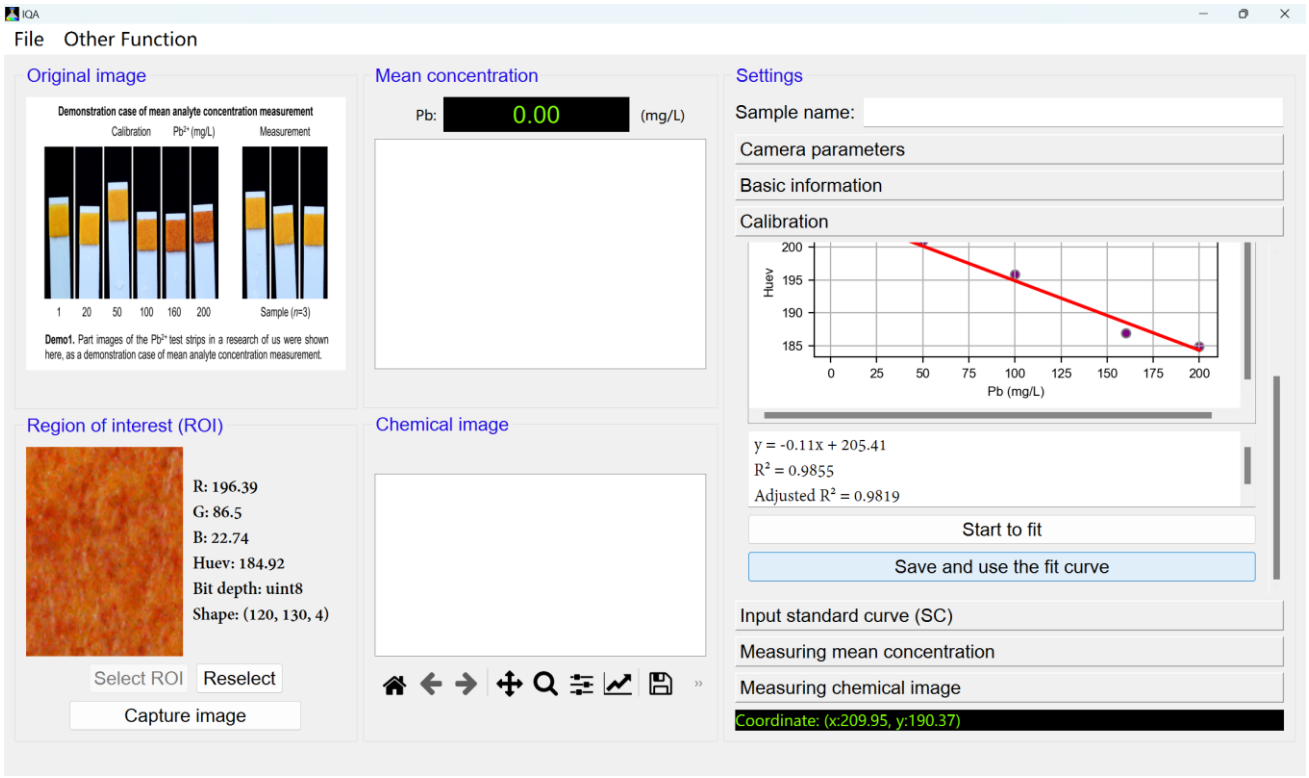


(3) Calibration. The <**Select save path**> button was clicked to set the path of saving calibration data. "Select ROI --- Input known concentration --- Click <**Add point**> button" steps were performed for every image used for calibration.

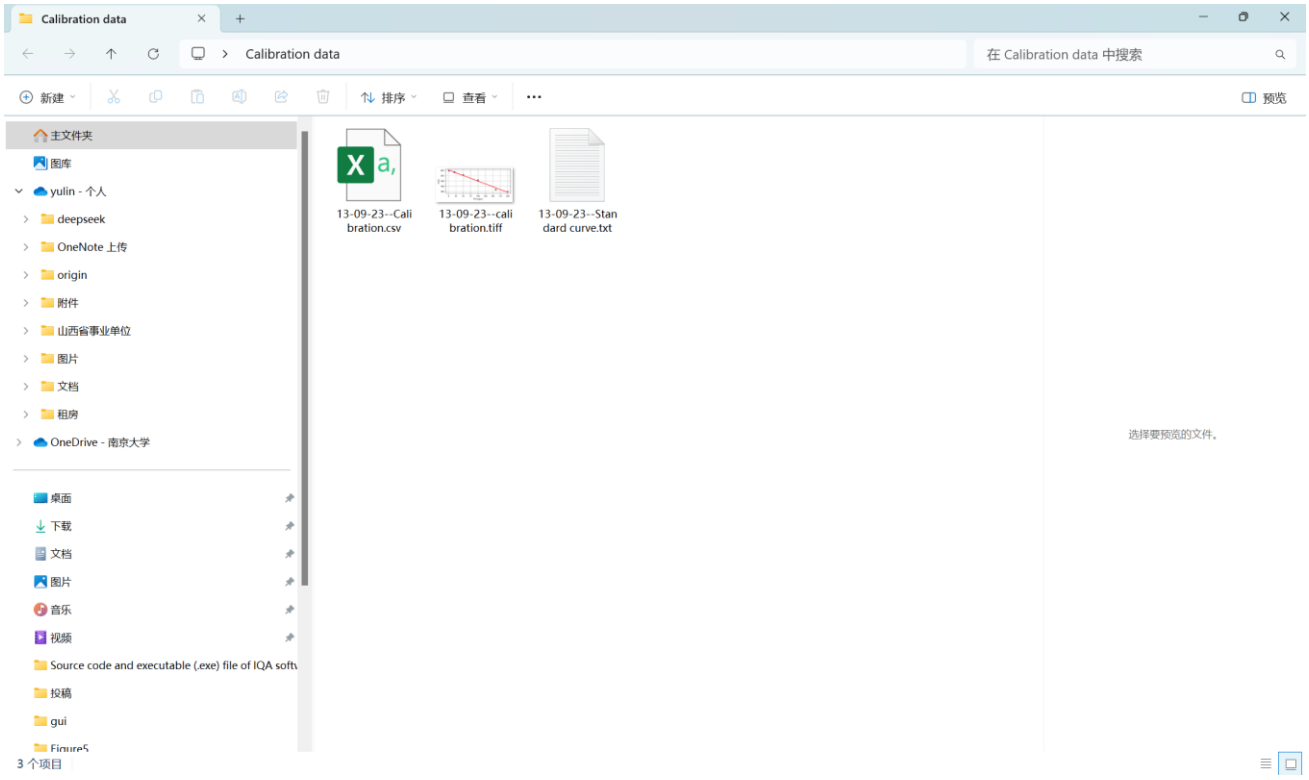


The <**Start to fit**> button and the <**Save and use the fit curve**> button were clicked.

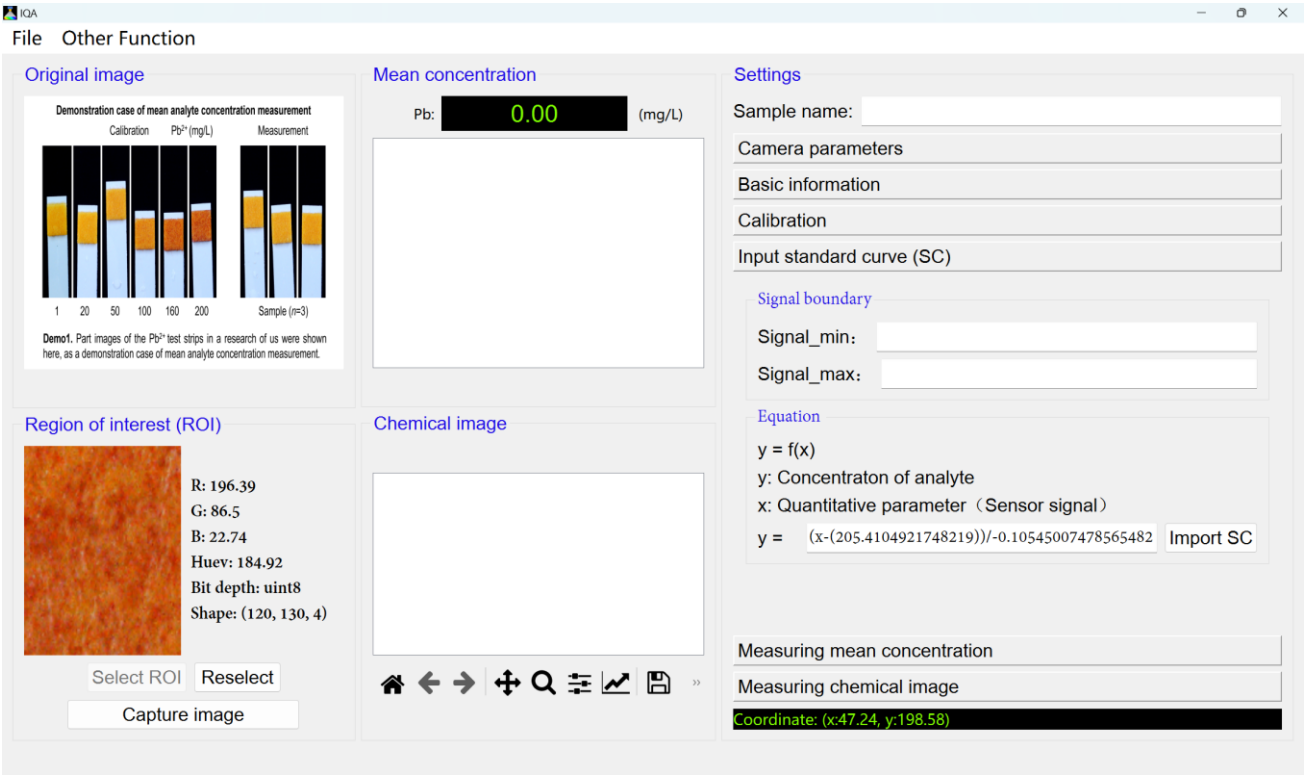




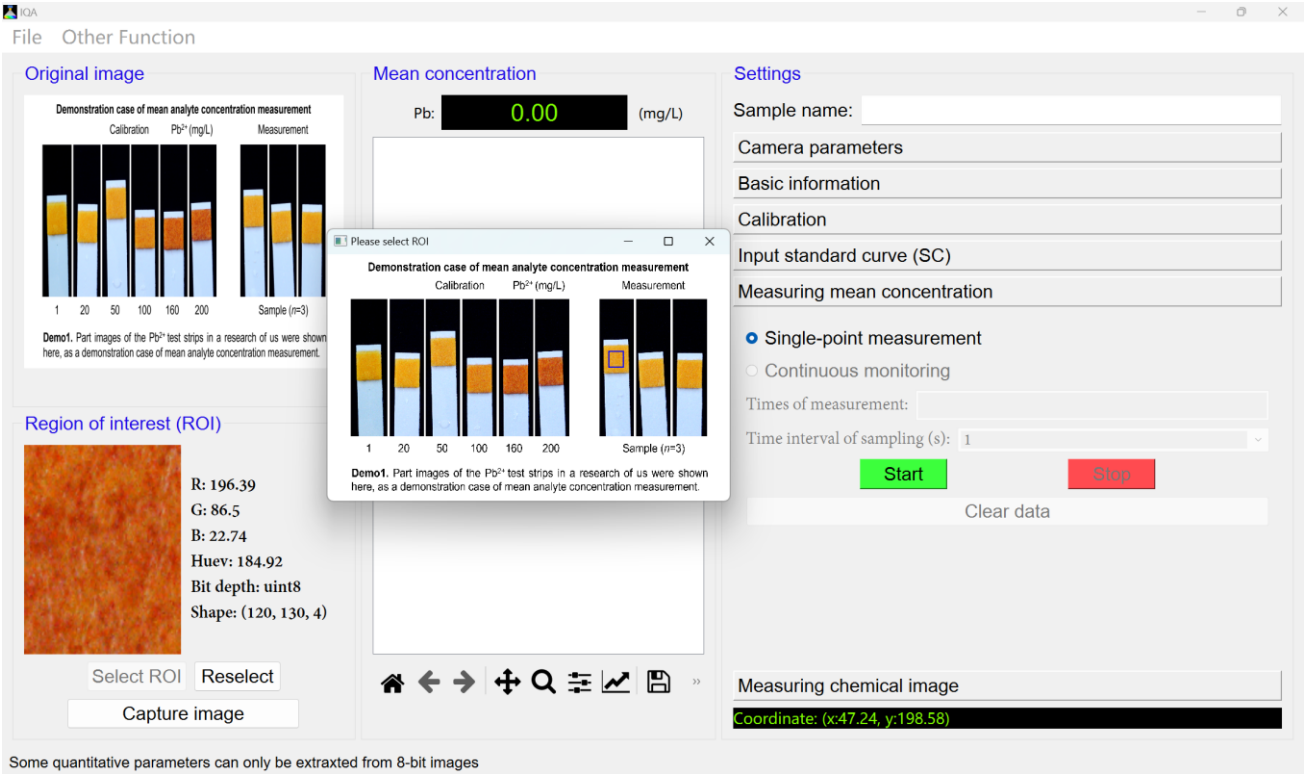
Then the calibration data (including original data and standard curve equation) will be saved, and the standard curve equation will be automatically applicated into the IQA.

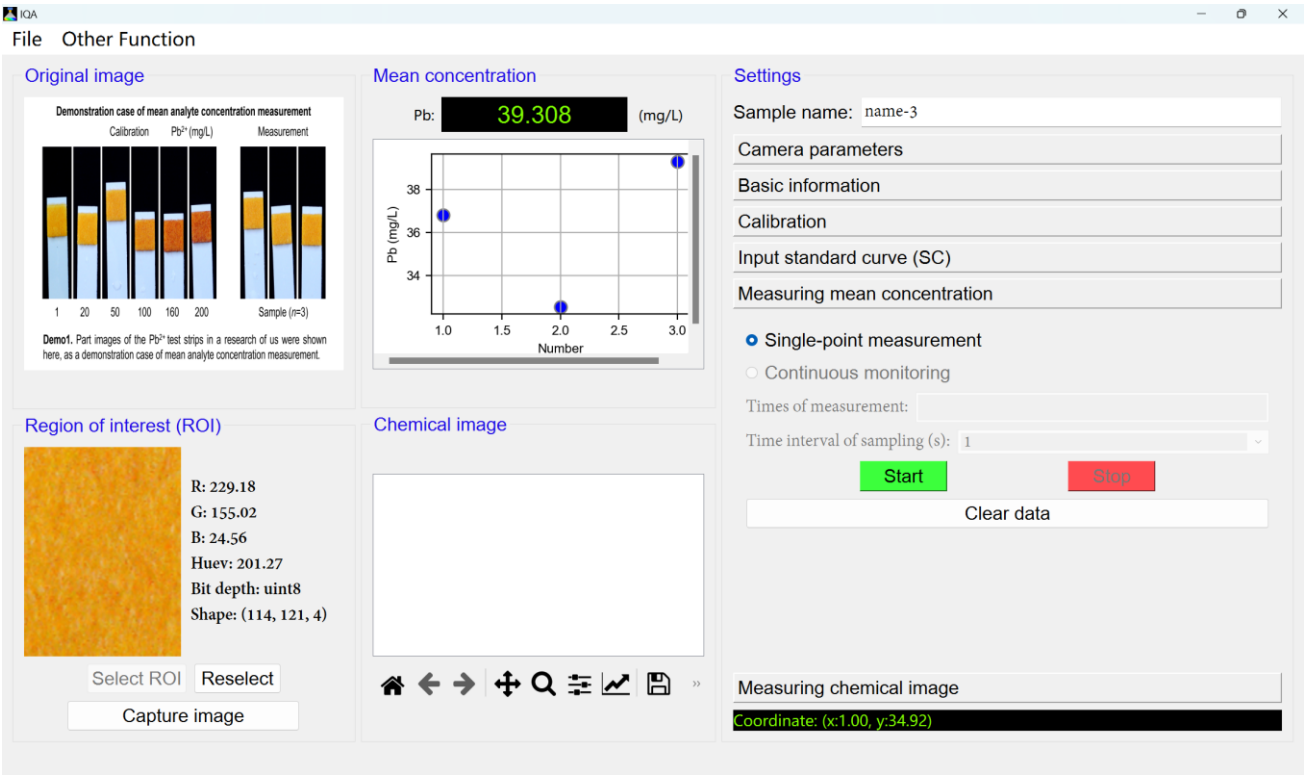


The standard curve equation is obtained from the fitted equation. Their independent variable and dependent variable are the opposite. The boundaries ('Signal_min' and 'Signal_max') of signals (quantitative parameter value) can be set if required. The signal values less than 'Signal_min' will be modified as 'Signal_min'. The signal values larger than 'Signal_max' will be modified as 'Signal_max'. The signal values will not be modified if 'Signal_min' ≤ signal values ≤ 'Signal_max'.

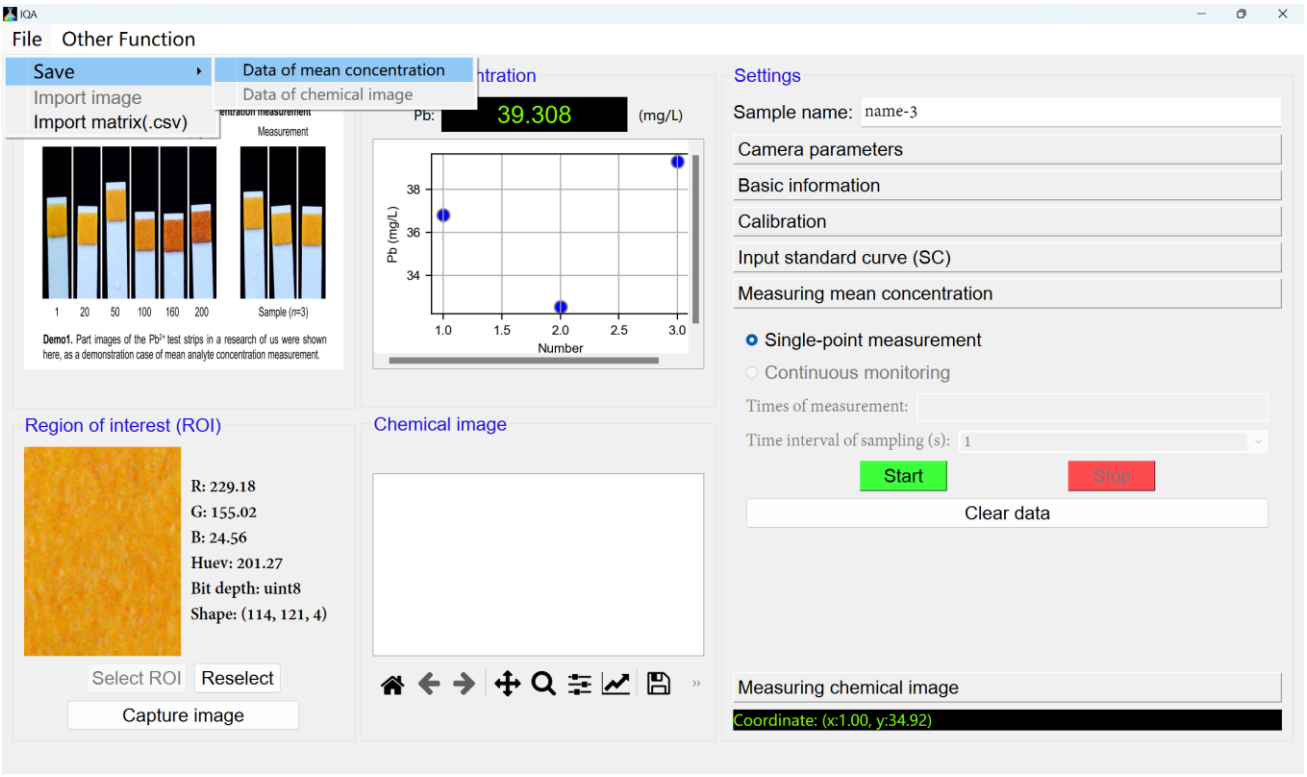


(4) Measurement of samples. “Select ROI --- Input sample name --- Click <**Start**> button in ‘Measuring mean concentration’ module” steps were performed for every sample.

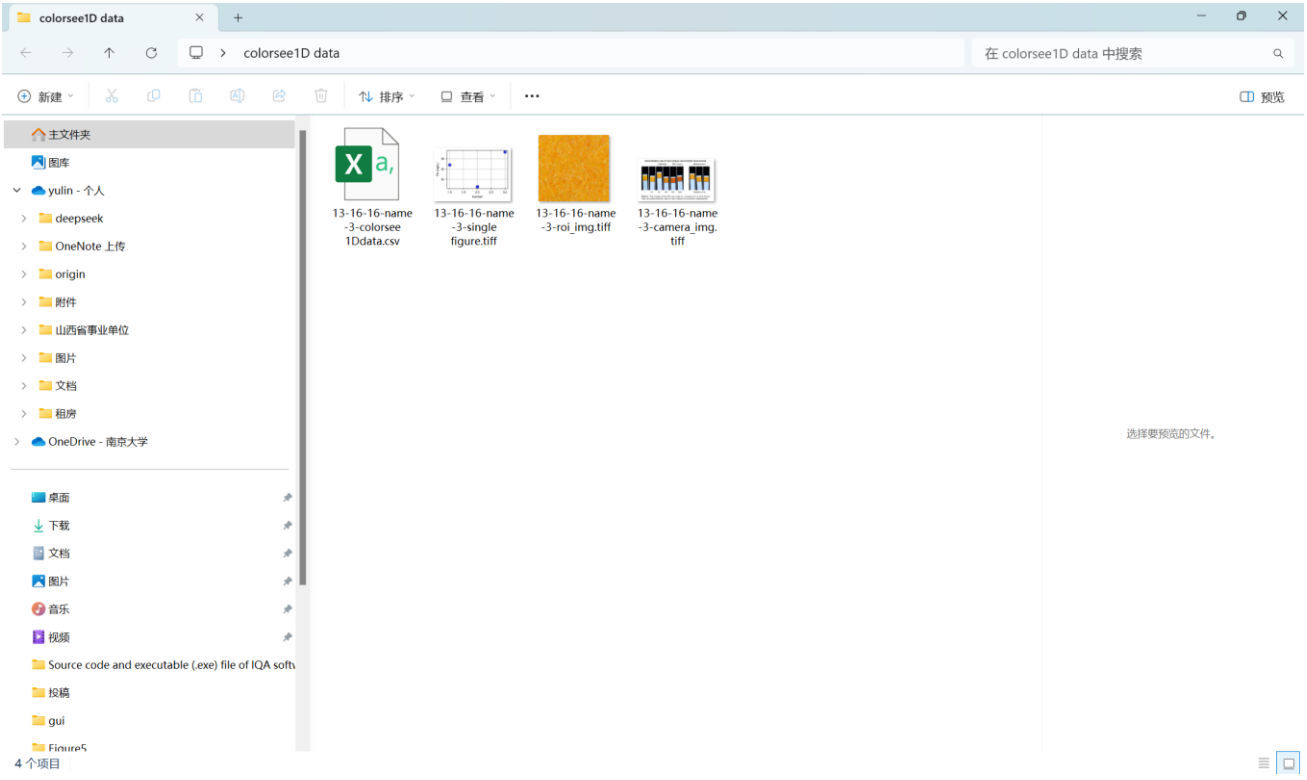




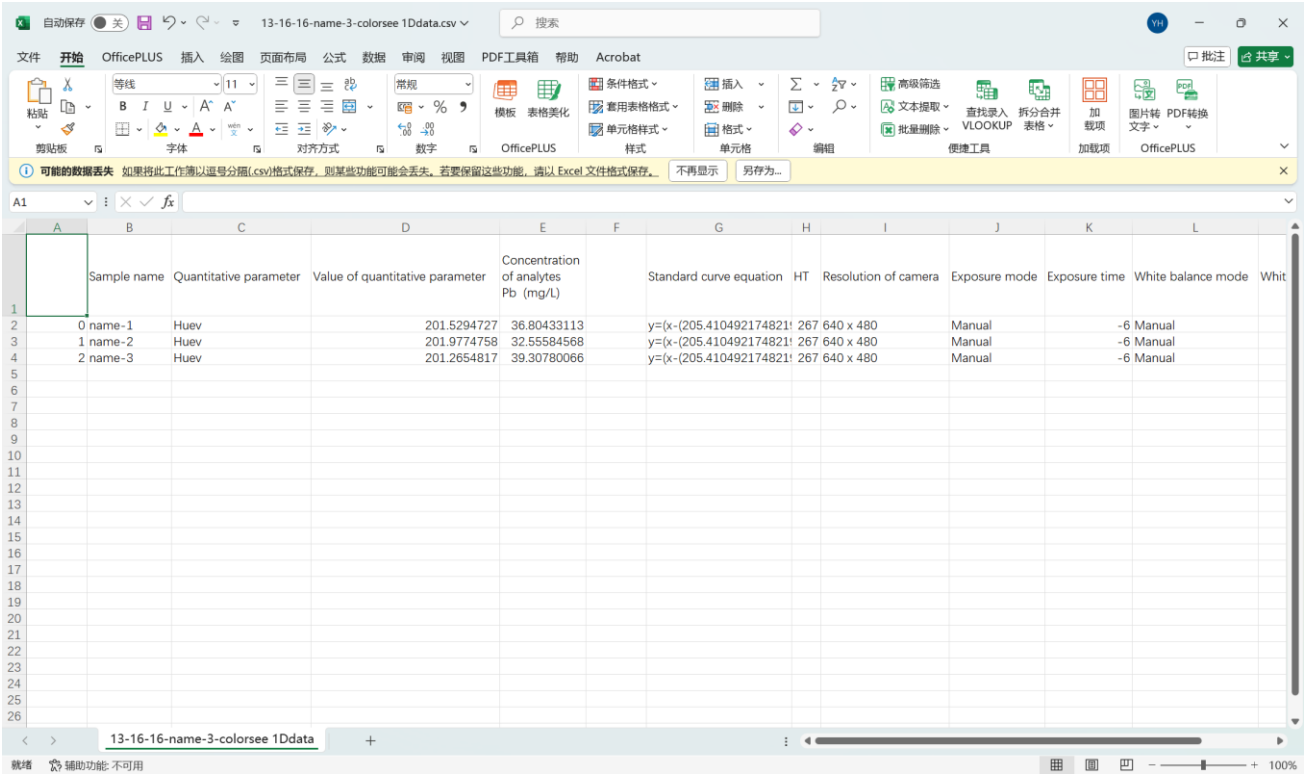
(5) Save of data. The <**File-Save-Data of mean concentration**> button was clicked.



Then the data will be saved into a folder named ‘colorsee1D data’, which is in the folder selected by the user.



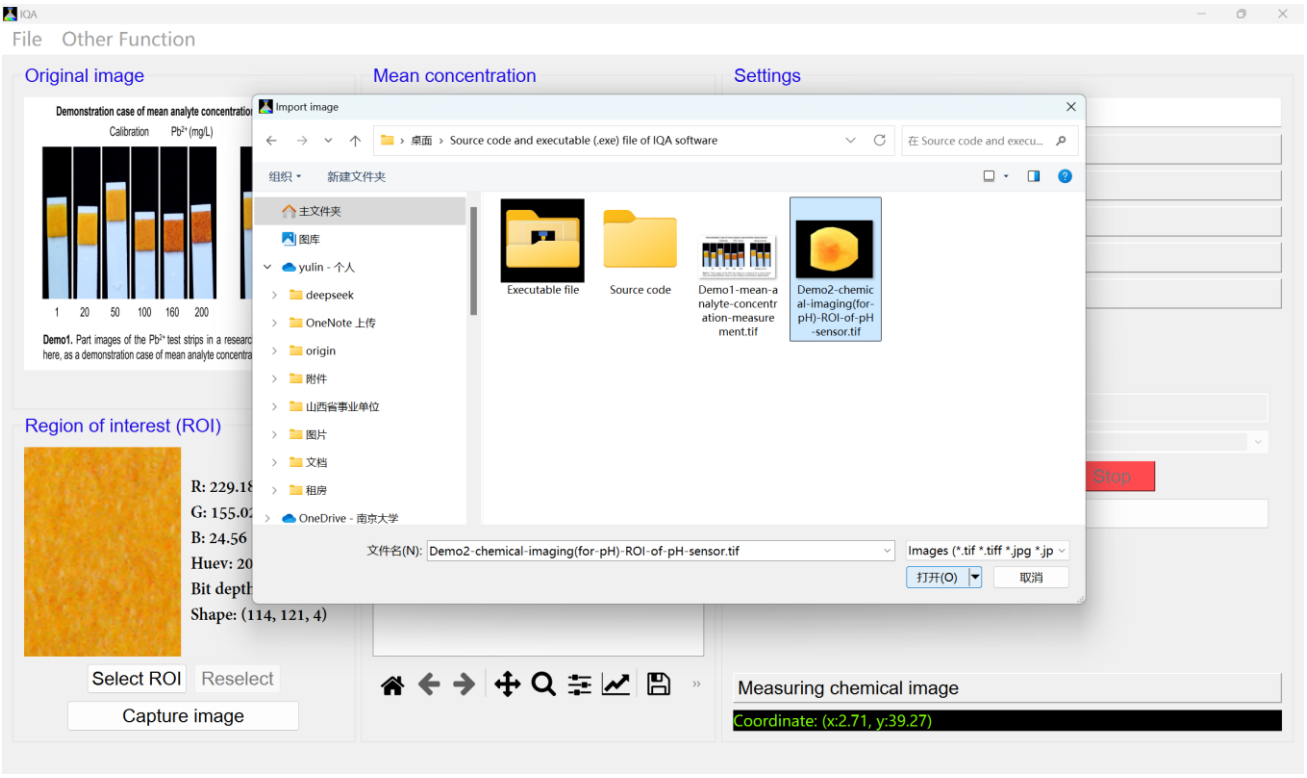
Some parameters during analysis were be also saved besides analyte concentration data of samples.



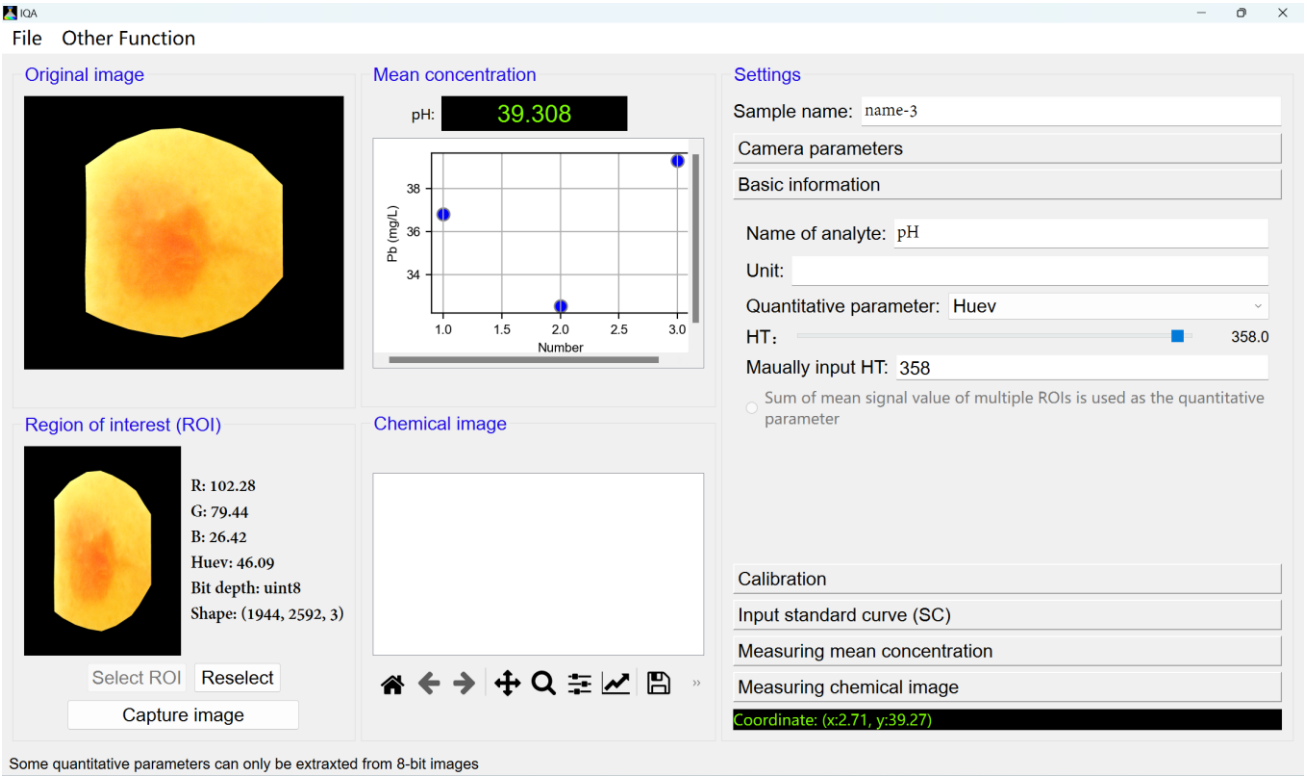
	Sample name	Quantitative parameter	Value of quantitative parameter	Concentration of analytes Pb (mg/L)	Standard curve equation	HT	Resolution of camera	Exposure mode	Exposure time	White balance mode	Whit
0	name-1	Huev	201.5294727	36.80433113	y=(x-(205.410492174821	267	640 x 480	Manual	-6	Manual	
1	name-2	Huev	201.9774758	32.55584568	y=(x-(205.410492174821	267	640 x 480	Manual	-6	Manual	
2	name-3	Huev	201.2654817	39.30780066	y=(x-(205.410492174821	267	640 x 480	Manual	-6	Manual	

3.Demonstration of chemical imaging

(1) Import the ROI image to be demonstrated. The polygonal ROI was selected by ImageJ software in this example. Therefore, ROI did not need to be selected after importing the image.



(2) Set basic information. The optimal HT is 358 for the universal pH test strip used in our research article.



(3) Calibration. The calibration method of chemical imaging is similar to that of mean concentration measurement. For the universal pH test strips, its complex response needs to be fitted by a piecewise function. The Origin software was used to perform this operation, using Hue_v (HT = 358) as 'y' and pH as 'x'. Then a piecewise standard curve equation that use pH as 'y' and Hue_v (HT = 358) as 'x' was obtained from it and manually input in a text (.txt) file. The text file was import into IQA by clicking <**Import SC**> button in 'Input standard curve (SC)' module.

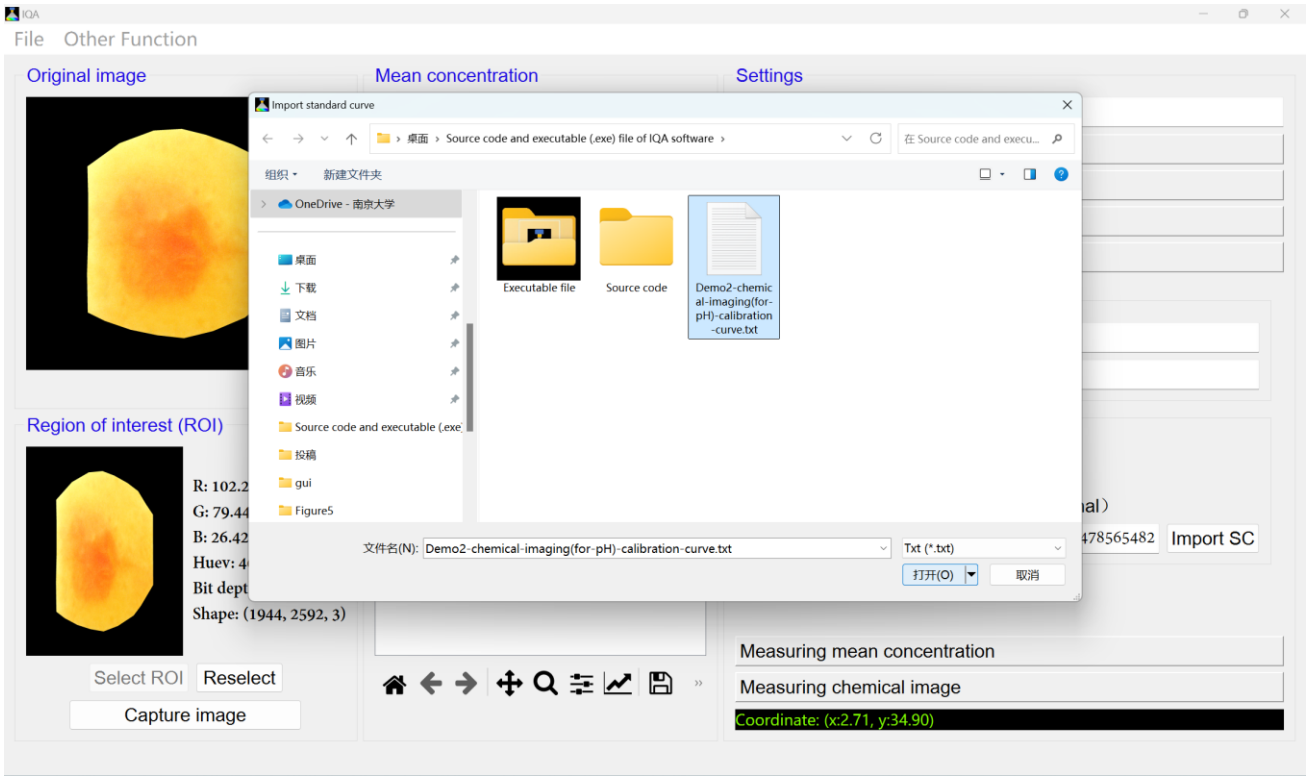
The writing format of standard curves are as follows. (i) If the equation only consists of addition, subtraction, multiplication and division, the '+, -, *, /' can be used as the operator. (ii) If the equation contains other operations such as logarithm and exponent, the functions in Numpy need to be used as operators (Table 1). (iii) The piecewise function can be written in the format as follows:

'$f_1(x)$ if condition1 else $f_2(x)$ if conditon2 else $f_3(x)$ if conditon3 else $f_4(x)$'.

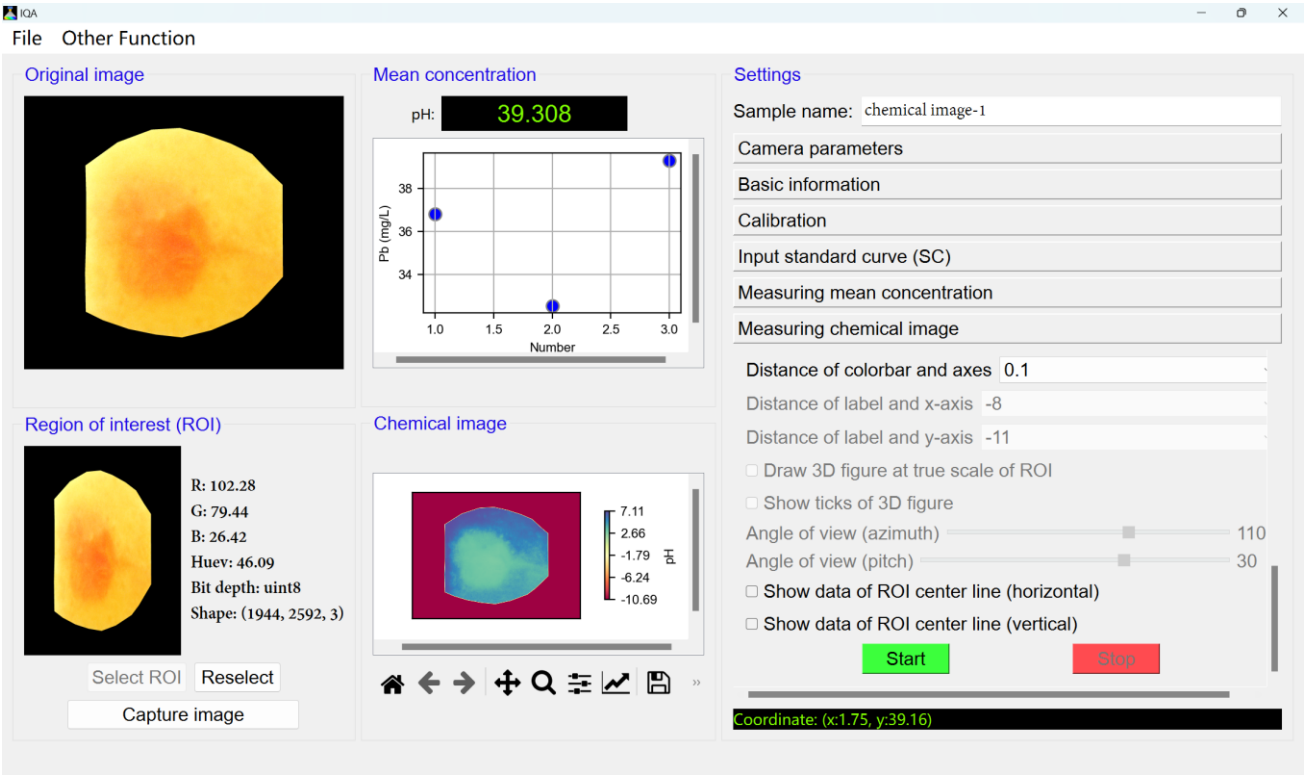
The last 'condition' (here is 'conditon4') does not need to be written. Otherwise, the software will report an error.

Table 1 | The input method of common mathematical operations

Mathematical operation	Format of writing	Mathematical operations	Format of writing
lgx	np.log10(x)	x^2	np.square(x)
lnx	np.log(x)	π	np.pi
$\log_a x$	np.log(x)/np.log(a)	e	np.e
e^x	np.exp(x)	≥	>=
x^a	np.power(x, a)	≤	<=



(4) Measurement of chemical images. The sample name was input firstly. The ‘colormap’ option was set as ‘Spectral’ and the <**Start**> button in ‘Measuring chemical image’ module was clicked. The Hue_v value of black area in ROI image is beyond the signal value range of standard curve so that the pH was calculated as -10.69 in this area.



To improve the contrast of pH image and ignore the pH values in black area:

the ‘vmin’ and ‘vmax’ were set as 2.50 and 7.11,

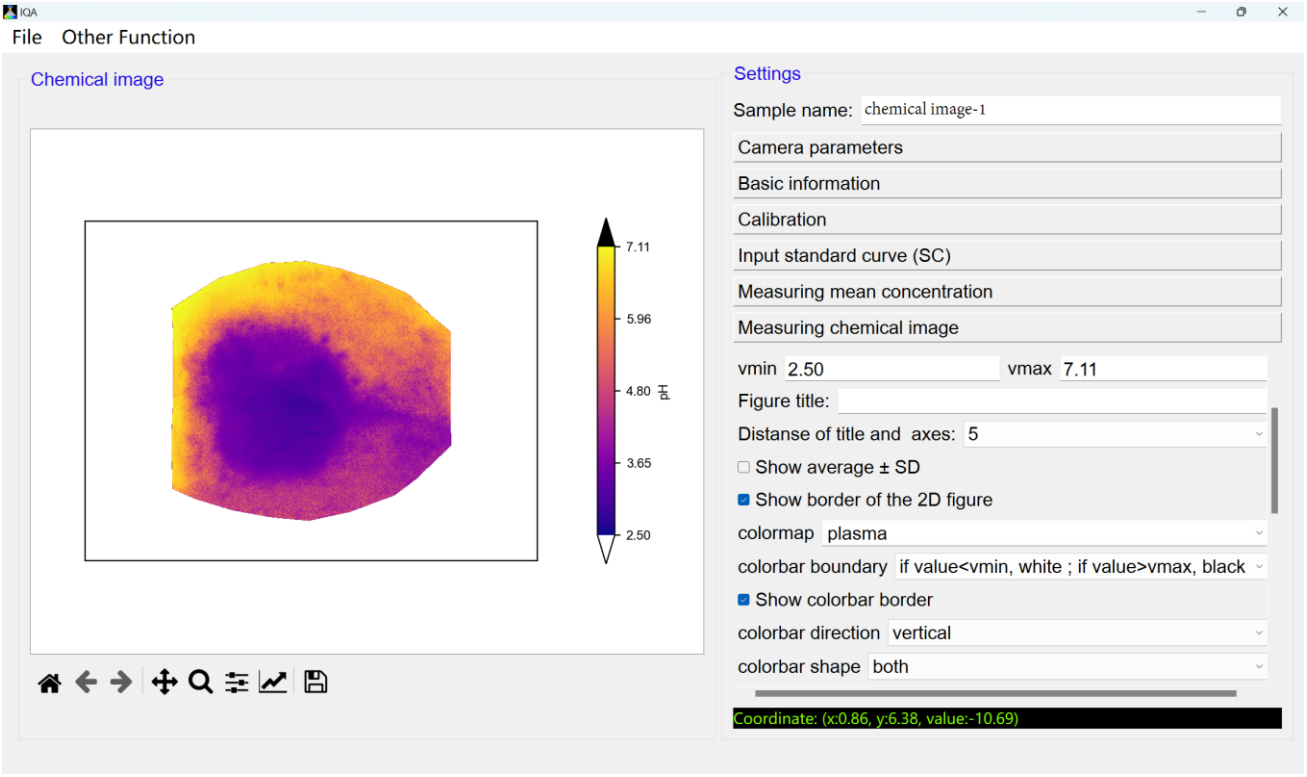
the ‘colormap’ was set as ‘plasma’,

the ‘colorbar boundary’ was set as ‘if value<vmin, white ; if value>vmax, black’,

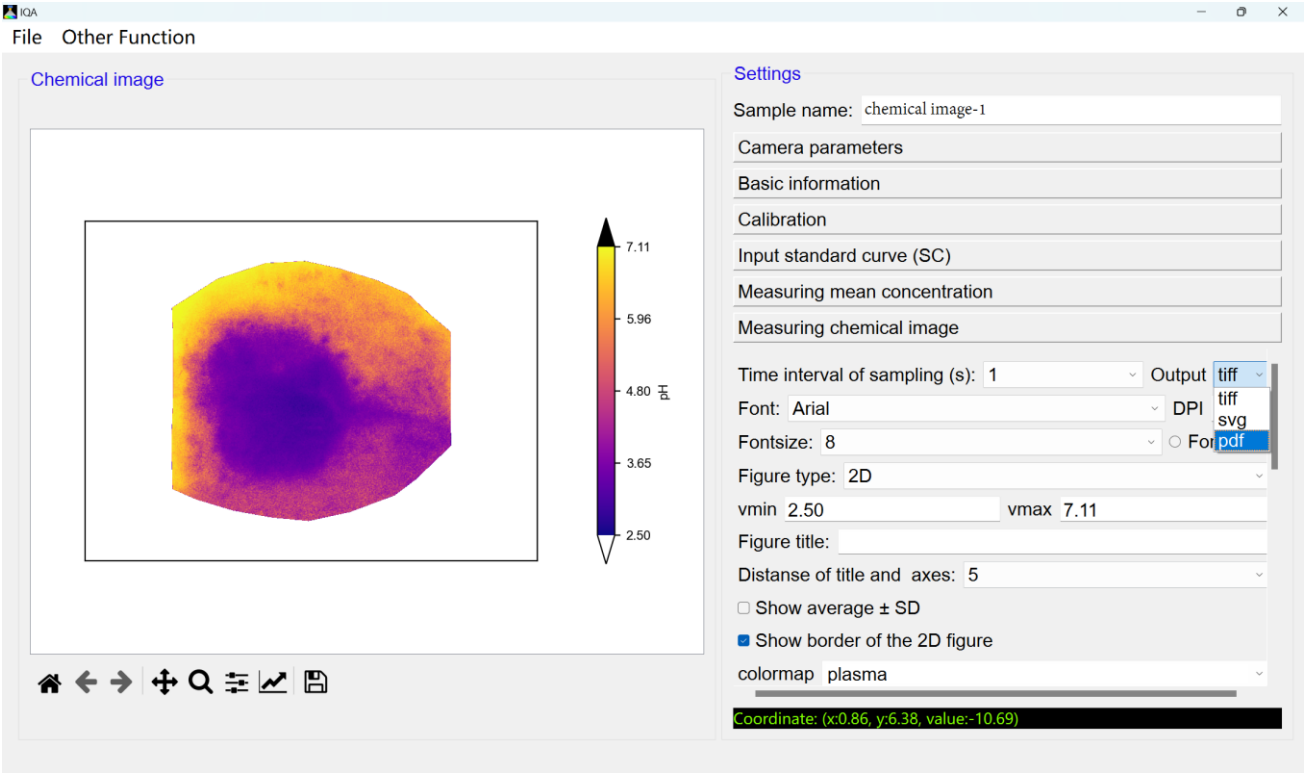
‘colorbar shape’ was set as ‘both’,

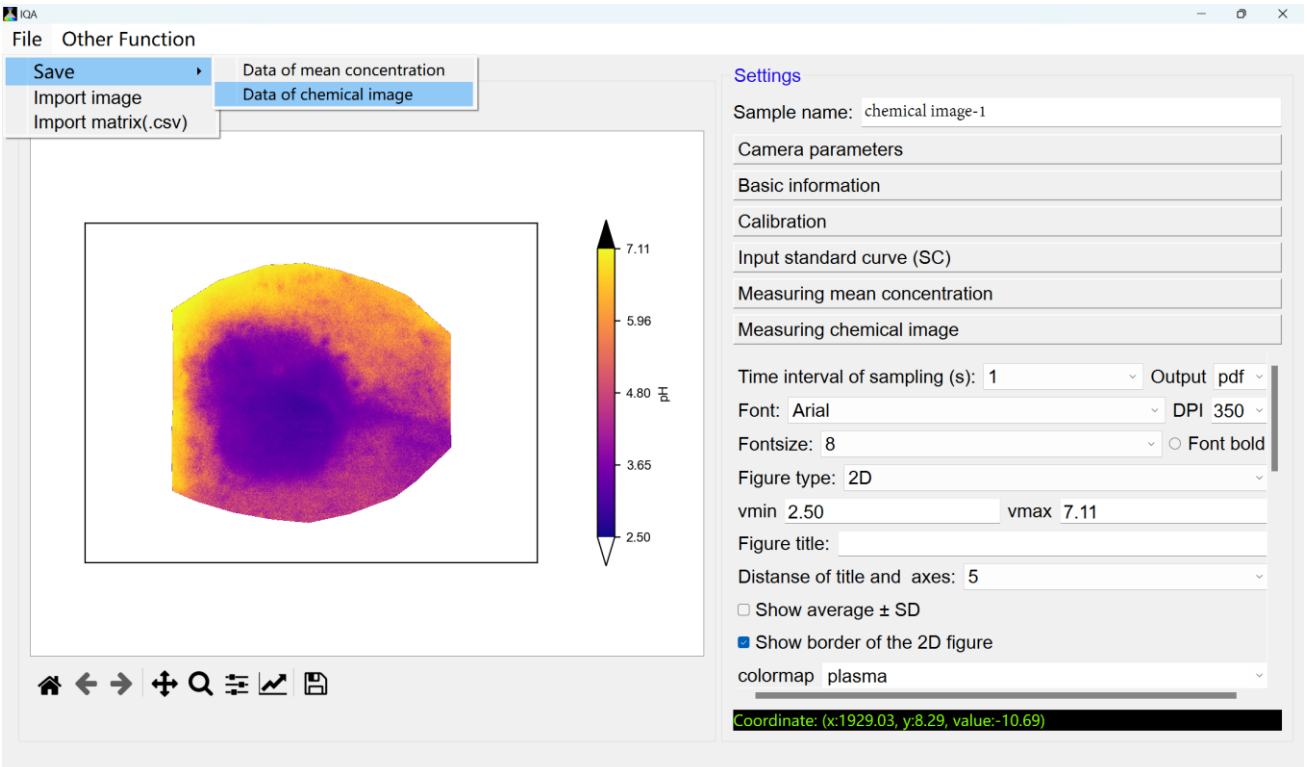
and the size of ‘Chemical image’ module was enlarged by dragging its border.

Then the pH image presented in our article was obtained after the <**Start**> button was clicked. The parameters used to adjust drawing styles of chemical images are very rich in IQA, which can be tried by users themselves.

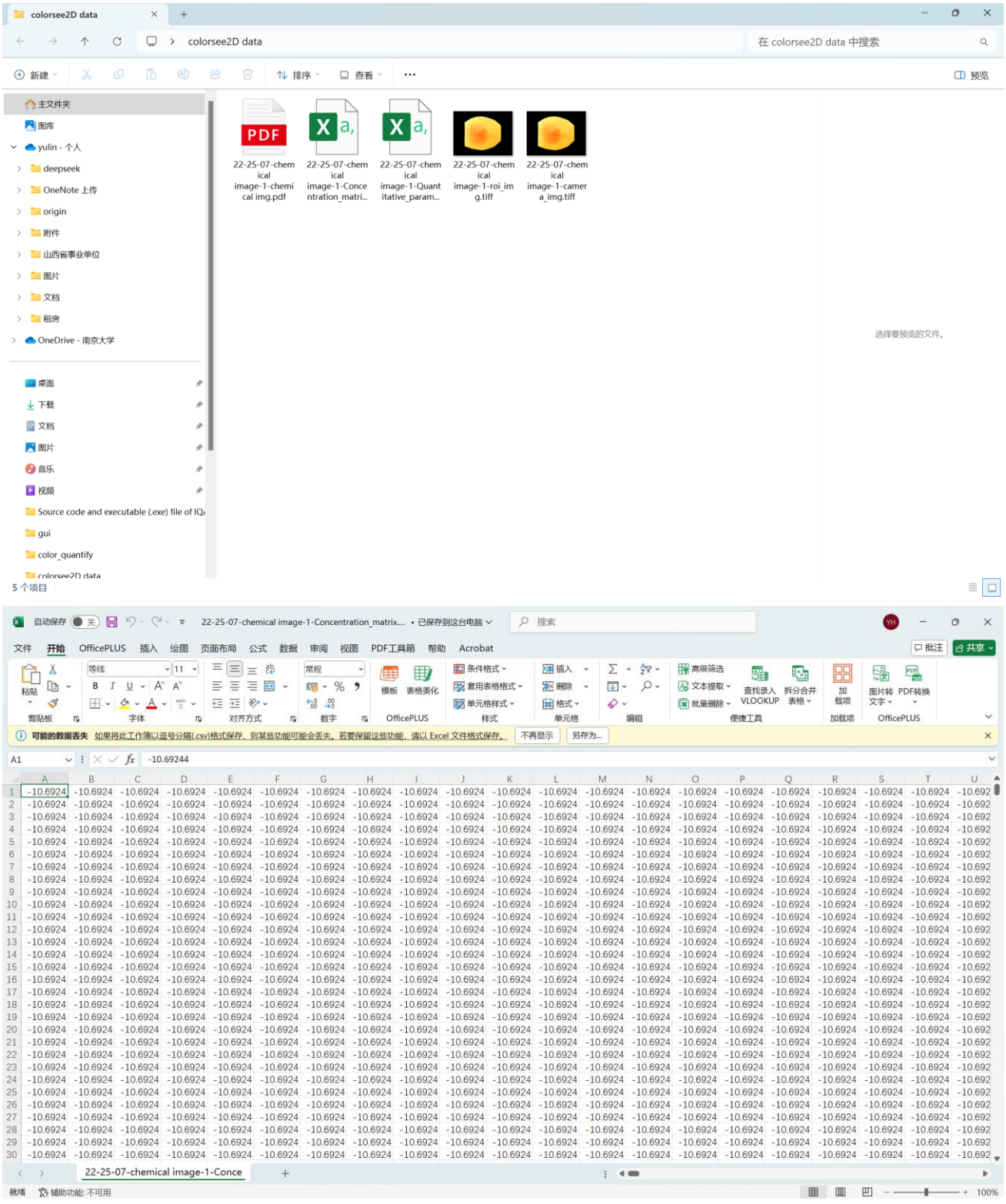


(5) Save of data. The <**File-Save-Data of chemical image**> button was clicked after the output format of figure was set as ‘pdf’ or ‘svg’ (the vector graphic with high clarity). Then the figures output by IQA will be allowed to be edited and typeset by Adobe Illustrator. The figures can also be output as high-resolution (>300 dpi) bitmaps of ‘tiff’ format.

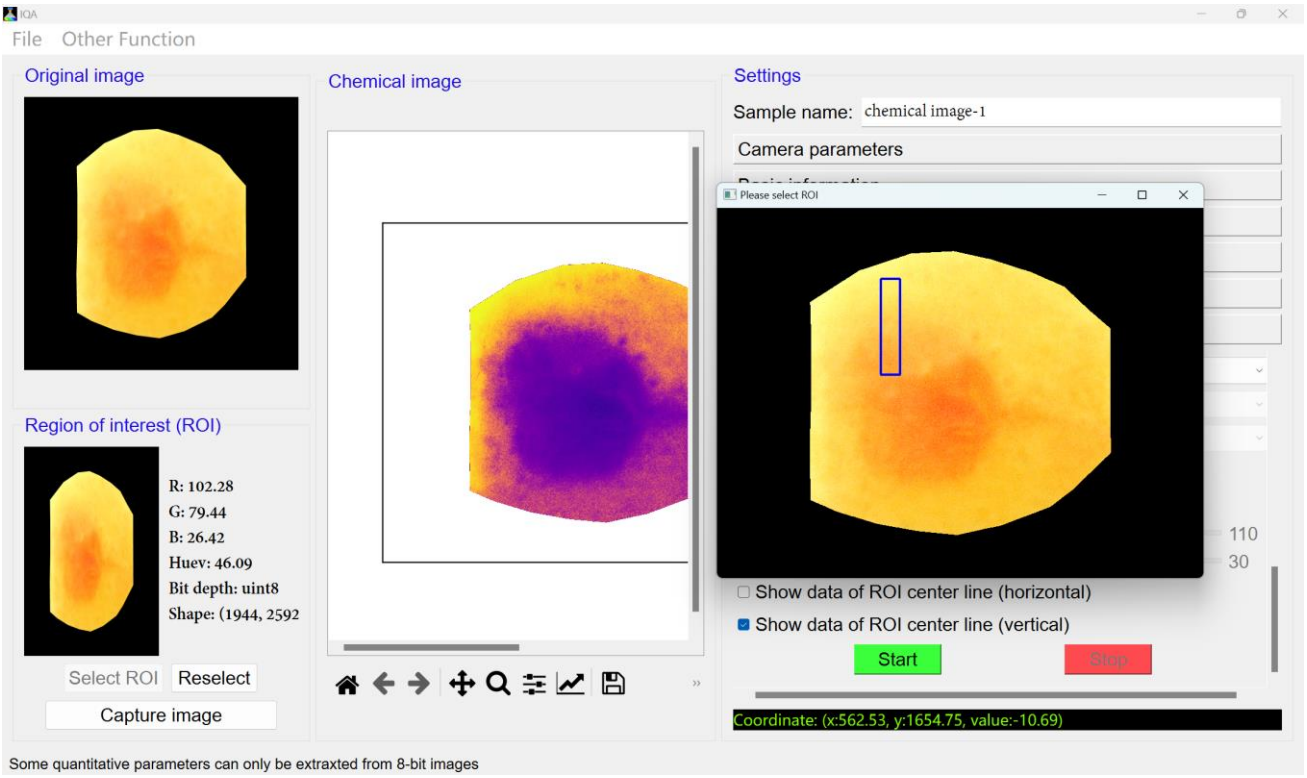


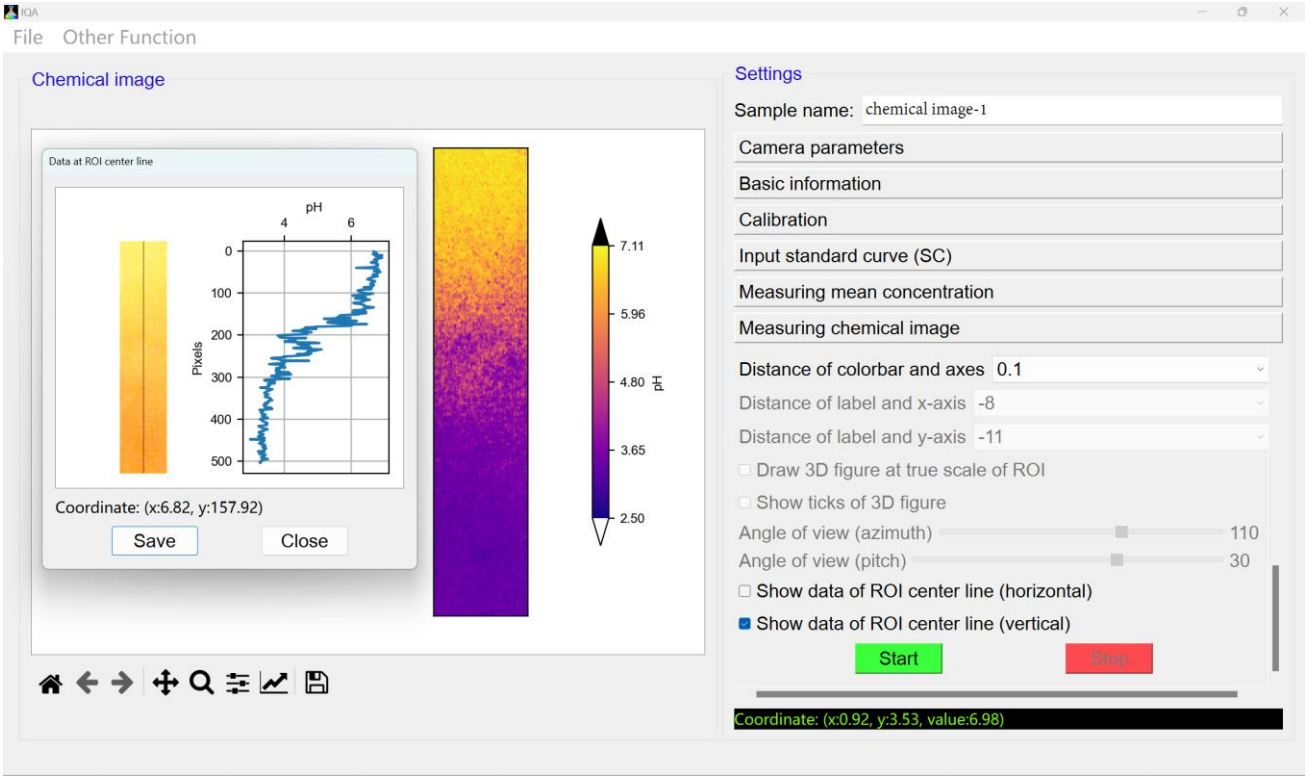


The chemical imaging data was saved in a folder named ‘colorsee2D data’. The ‘.csv’ files used to save chemical image matrix and quantitative parameter matrix are provided, which allows users to perform other analysis according to the original matrix data.

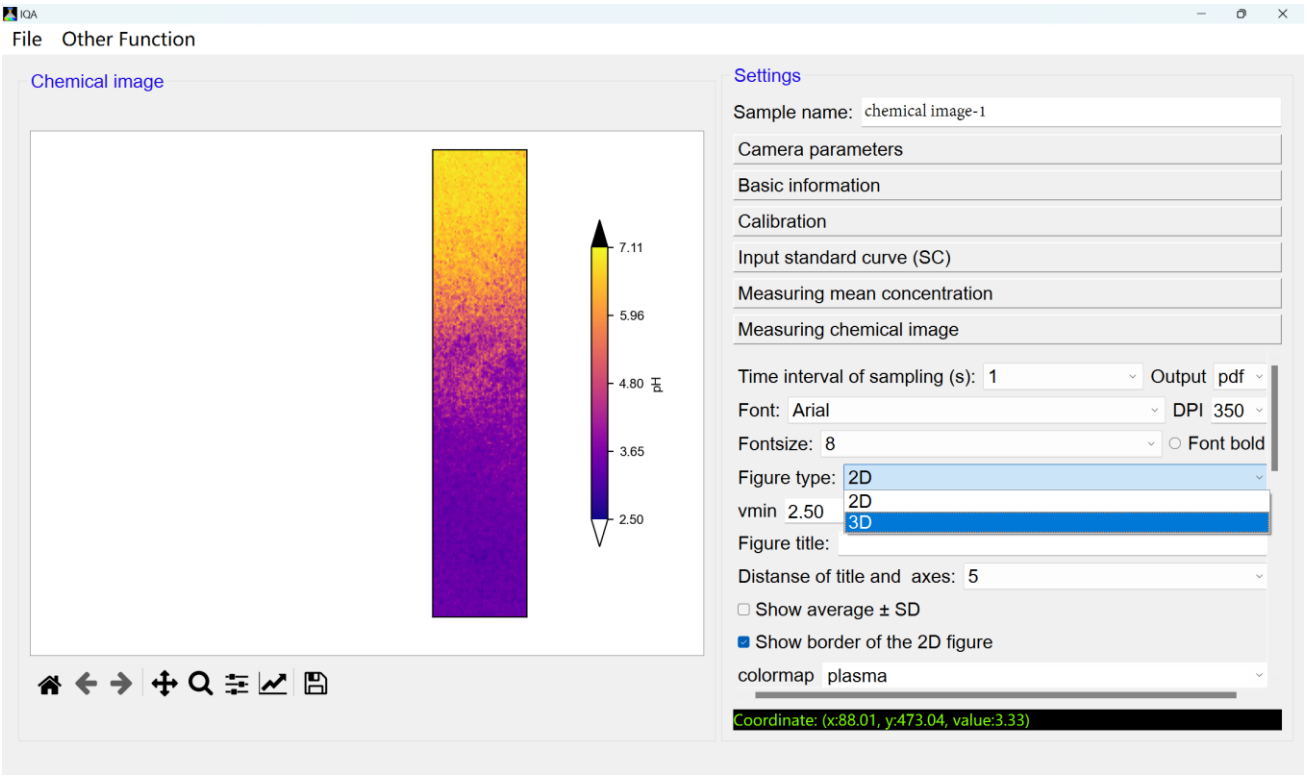


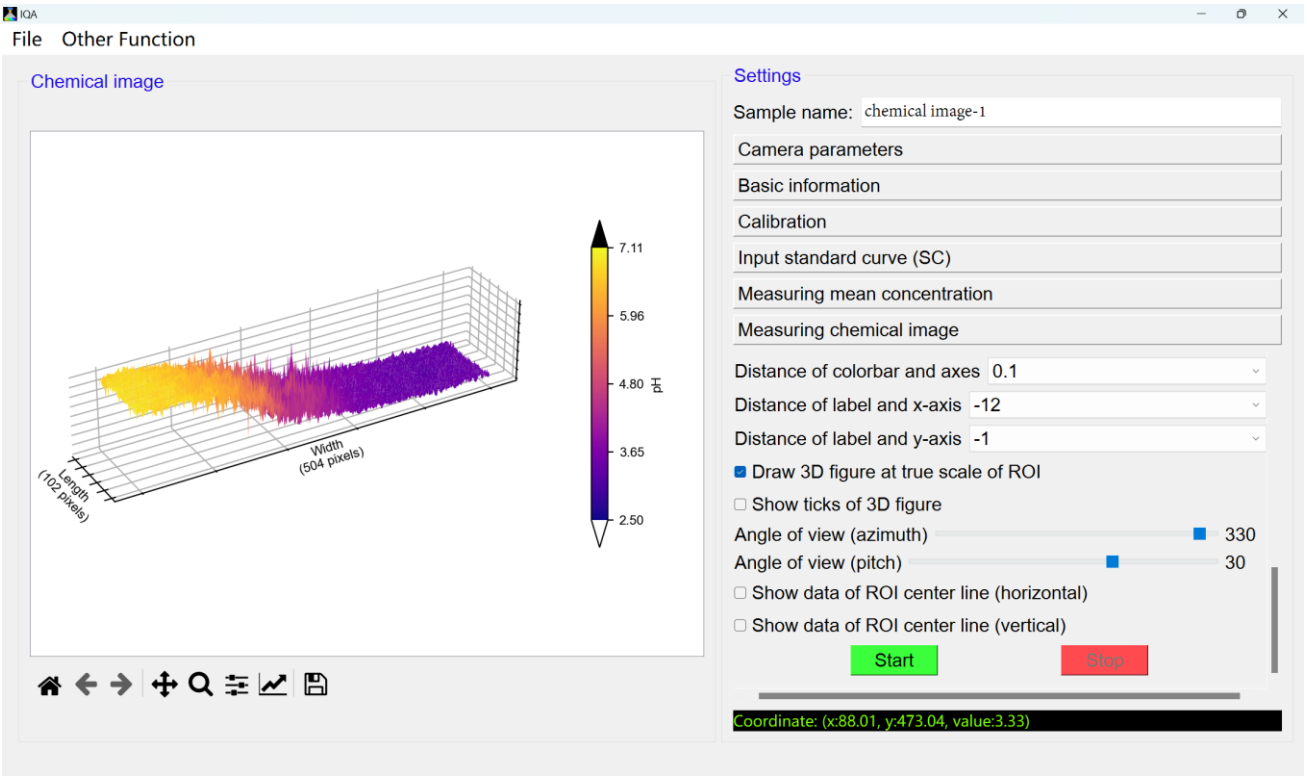
The data in ROI center line (horizontal or vertical) can be extracted by selecting the option of ‘Show data of ROI center line (horizontal)’ or ‘Show data of ROI center line (vertical)’ before clicking <**Start**> button.





If ‘Figure type’ is set as ‘3D’, the 2D distribution of analytes can be presented in 3D format. Sometime this format is more intuitive. The view angle of 3D figure can be adjusted for optimal visualization effects.





4. Other functions of IQA

Overall, the functions of IQA focus on measuring mean analyte concentration and chemical imaging. Here, we only demonstrated the processing of images stored on a computer's hard drive. The processing of images read directly from a camera or from video files is similar. In addition, users can try functions such as continuous monitoring of mean analyte concentration, capturing chemical imaging videos, recording videos from a camera, plotting 2D/3D pseudo-color images from imported '.csv' matrix files, and switching quantitative parameters (IQA also integrates multiple quantitative parameters based on existing color spaces).

IQA is a powerful and user-friendly digital image colorimetry software. In the future, we will continue to update its features and correct any potential bugs.