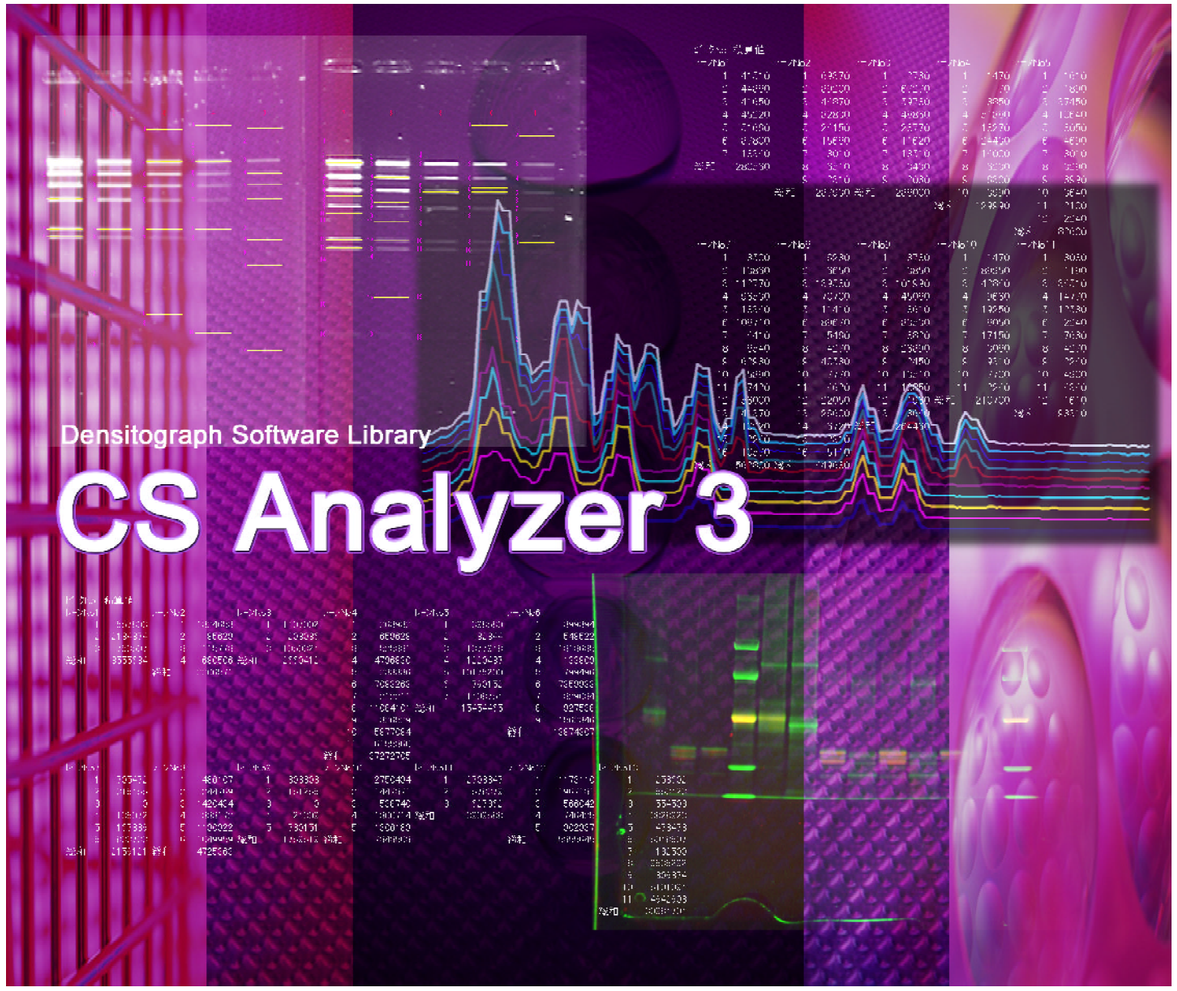
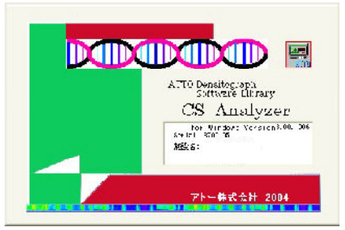


ATTO Densitograph Software Library

CS Analyzer 3 for WindowsXP

バイオ日本を支援する純国産ブランド

CS Overview

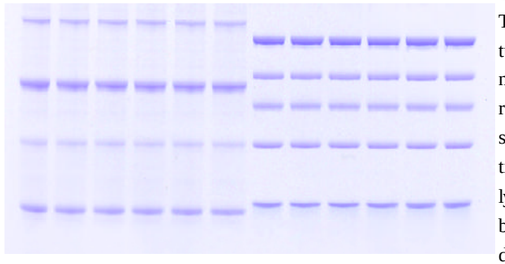


CS Analyzer is an image analysis software developed and sold by ATTO (HQ:Bunkyo-ku Tokyo) which enables fixed quantity analysis for bands and spots dot blot patterns, as well as one-dimensional electrophoretic patterns of proteins, nucleic acids etc.

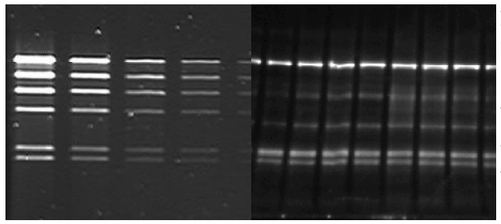
CS Analyzer is equipped in the ATTO's "Light-Capture" series of cooled CCD camera systems Windows XP. It not only analyzes the images but also captures images by controlling LightCapture.
This software can be purchased as a standalone product, so it can be used in image capture/ analysis systems when combined with the gel photographing apparatus "Print Graph".
Main Functions
*Image analysis: Molecular-size calibration, density fixed quantity, etc.
*Image input: LightCapture, Print graph (video input and network connection), scanner input.
*Image conversion:Image composition, resolution change (enlarged twice or reduced 1/2), inversion, rotation, and grayscale (No. of bits) change, etc.
*Display image adjustment: Contrast adjustment and pseudo-color display.
*Compatible image formats: JPEG, BMP, TIFF (8 bits to 16 bits), CCD (Light capture), IS (Hight-Resolution: Print graph).

What kind of samples can it analyze?

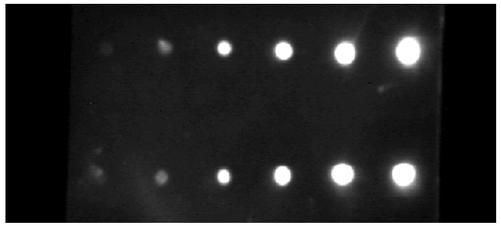
The CS Analyzer measures, the electrophoretic pattern of proteins and nucleic acids, and estimates molecular weight(size) of each band and measures the density (= amount). At that time, the molecular weight marker and a marker for fixed quantity of standard substance are required. Additionally, it has image conversion and contrast adjustment functions to suit the detection method. An example of a target pattern for analysis is shown below.



SDS-PAGE
This is a typical electrophoretic method for proteins. Images captured using a gel photographing apparatus (monochrome), image scanner or digital camera can be measured. For measuring density, we recommend capturing an image with a gel imaging device. When the shading compensation function of the CS Analyzer is used, irregularities in the penetration light source can be reduced. The image is analyzed after the absorbance conversion is performed. The density can be measured when there are correlations in the dye method and the density of the protein. (Note: there is may be differences depending on smpale).



Fluorescent stained gel chemical luminance detection pattern
Fluorescence and the luminescence-patterns captured using an imaging device such as LightCapture, Print Graph, etc. can be analyzed. By adjusting the contrast on the PC monitor (contrast only on display is changed without changing data), faint bands become easier to check. A wider dynamic range can be obtained in fluorescence and luminescence patterns compared with pigment staining.



Dot Blot Pattern
Dot blot patterns of protein and DNA can be quantified. When using chemical luminance device such as LightCapture luminescence detection provides excellent fixed quantity data. It is possible to measure by enclosing the dot with spot measurement in the measurement area.

Software Specifications
ATTO CS Analyzer 3- Supported OS, Windows XP/ 2000, Excel etc. Microsoft Corporation.
Function
Image Input
LightCapture control
-Imaging control function of ATTO LightCapture
-Auto exposure function/ No limitation of images savable under continuous image taking(setting is necessary)
-Video input: Gray image/color image can be captured using a PC equipped with video a interface, Unevenness of white light transilluminator can be reduced by correction of shading.
-Scanner input: Scanner supporting a TWAIN driver can be used as plug-in(supporting 16/48 bit input)
-NetWork input: Images in AE-6905CF Image Saver of Print Graph can be transferred.
Open
*8bit(256 grayscale):BMP/ JPEG/ TIFF/ IS (Print Graph:HighResolution)
*10bit(1024 grayscale):TIFF (Print Graph: 10bit)
*12bit(4096 grayscale):CS file(LightCapture: ccd)/ TIFF (Print Graph:12 bit) /Chain file(LightCapture: cha)
*14bit(16384 grayscale):Cs file(LightCapture: ccd)/Chain file(LightCapture: cha)
*16bit(65536 grayscale):TIFF/ CS file(LightCapture: ccd)/Chain file(LightCapture: cha)
Reanalysis open: Data for reanalysis that is saved by CS analyzer (extension: can)

Measurement function
*Molecular size estimation: Calibration curve is created from the degree of movement of molecular-size maker band and molecular-size.
*Densitometry: Digitalization from intensity of band, detection of peak position (at zone densitometry), (electrophonetic) mobility, etc.
*Fixed quantity function: Molecular-sizequantitation using molecular-size estimation, Relative density quantitation from measurement value of digitalized band.
*Measurement method: Zone-densitometry, specified area densitometry, spot measurement (Lane distortion correction, background correction, peak range correction).
*Scale setting: Scale can be set in an image using a line (used as a scale for full size printing).

Saving function
*Image save: 8bit image save (format: BMP, JPEG, TIFF) converted image, measured image, profile image etc., 12 to 16 bit image save (format: CCD) format dedicated to CS file, 16bit image save (format:TIFF) 16bit TIFF image save, 12 to 16bit Chain file (format:cha) saving the image captured by LightCapture.
*Text save
*Measurement result and profile data can be saved as test.
*Data save for reanalysis: Image conversion, measurement area setting and measurement result are saved together at termination of measurement, By opening Reanalysis, data can be displayed again under the condition at termination of measurement.

Image convert
*Pseudo coloring: Brightness distribution of monochrome image can be displayed in dummy color.
*Contrast display adjust: Contrast on monitor display can be changed freely without changing brightness data.
*Absorbance convert value: Conversion function for measurement of an image such as CBB stained gel, in which absorbance becomes an index for density.
*Change the image resolution: Printing resolution is improved by doubling the number of pixels of the image(both horizontal/vertical direction). It is acceptable to change the same image multiple times(file size becomes 4 times larger each time).
*Image composition: Composition of luminance pattern and pre-stained marker image(image calculation/subtraction function), RGB composition function, Shading correction(reduction of unevenness of white transilluminator). etc.
*Full scale printing: Printing magnification is adjusted automatically so that the set scale becomes full scale.

PC environment
CPU: Pentium III or above
Memory: 256MB or over
HD capacity: More than 1GB is recommended.

*CS analyzer 3 software is dedicated for use under WindowsXP.
*It does not support Macintosh PC OS.
*CS analyzer software is equipped on [LightCapture] as standard.
*Analysis including band measurement and molecular-size assumption is used to measure bands, etc. that have higher intensity than background.

For pigment stained patterns, analysis is performed after absorbance conversion.

NEW Image analysis system

Quantitive analysis systems for fluorescence stained gels and pigment stained gels

AE-6943V-FX Densitograph

Sample that can be imaged
- Fluorescent stained gel (UV excited)
- Pigment stained gel (CBB, silver stain, etc.)
- X-Ray film
- Other visible samples

Analytical functions
- Density fixed quantity of fluorescent pattern and pigment stained pattern
- Molecular size estimation (A molecular size marker is necessary.)

Product configuration
-AE-6933FXCF-U Print Graph (1 wavelength UV)
-PC system (Windows)
-Analysis software / CS Analyzer Windows version
-Accessories and instruction manual

Option
Epi-UV option kit for FXII
Two wavelength UV Transilluminator (please consult us)
AE-6935 Visirays (light source for LED fluorescence excitation)
*Please consult us for details.

NEW New affordably priced set of luminescence & fluorescence & visible light imaging devices

Chemiluminescence imaging & Fluorescence imaging & Visible light imaging & Analysis Software - All integrated into ATTO COMBO

AE-6981GXCP ATTO COMBO II

AE-6981FXCP ATTO COMBO II

The ATTO COMBO series is an integrated system that consist of a chemiluminescence imaging device [LightCapture], a gel image-capturing device [Print graph], a monitor, a printer, and a image analysis software. The whole system is available at a reasonable price.
ATTO COMBO offers a systematic tool for capture, storage, and analysis of images generated by chemiluminescence, fluorescence, and visible light (CBB or silver stained gel , etc.) in research and development.

Imageinputfunction

- Light Capture series

When the LightCapture and personal computer are connected, control (image taking/ image capturing) is facilitated with CS Analyzer 3.

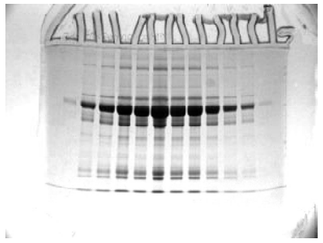
NEW ! AutoExposure (auto exposure imaging)
Appropriate exposure time is calculated automatically and image is taken.
NEW! SemiAuto Exposure (arbitrary time imaging)
Three arbitrary exposure times are selected and images are taken continuously.
* Single (Single imaging)
* Repeat (Repeated imaging)
* Sum (Integration imaging)
* AutoSum (Integration imaging with saturation detection function)
* Read VRAM (video memory image copying function)
-*Shading compensation * function(for the white light source when taking an image)
This function corrects irregularities of the white light source when taking images of CBB stained gels and such.

LightCapture and PC system

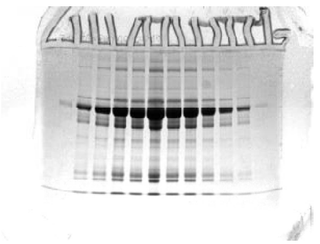
-Print Graph series

By connecting a personal computer with an interface board for video composite input PrintGraph, images can be input using CS Analyzer 3 (video input function). When the AE-6905CF Image Saver and a PC are connected as a network, images saved in the Image Saver internal memory can be captured network connection function).
*AE-6905CF is implemented for the AE-6931FXCF/ 6932GXCF Print Graph as standard.
* Network connection(AE-6905CF and the personal computer connected as a network.)
* Shading compensation * function(for the white light source taking an image)
→This function corrects irregularities of the white light source when taking images of CBB gels and such.

Print Graph



Before shading compensation



After shading compensation

* : Shading compensation is performed using 2 images; the gel image photographed under defined conditions and the image of the light source only having removed the gel after photographing.

- Image scanner

Images can be captured by controlling a scanner supporting Twain drivers as a plug-in.
* Scanner driver is used for capture (supporting 48bit color/ 16bit gray)

Image scanner

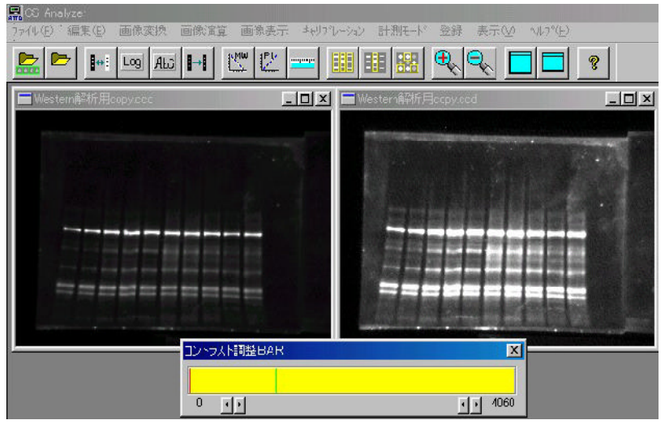
CS Analyzer image conversion function

Contrast adjustment function for displayrd Image

The CS Analyzer can handle 10 to 16bit images grayscale than normal 8bit files(BMP, TIFF, JPEG, etc.).

In the case of 10 to 16bit images, it is often not easy to see bands easier to see. Since the contrast adjustment doesn't change the intensity of the image, the same measurement result is obtained regardless of the application of adjustment.

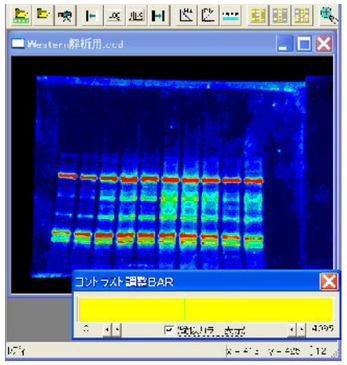
It is also possible to convert the image with adjusted contrast into a TIFF, BMP, or JPEG 8bit file and save it.



Contrast of appearance is changed. Intensity data of the image does not change, therefore, appropriate measurement results can be obtained when the image is analyzed after adjustment.

Pseudo color display

When contrast of displayed image is adjusted with the CS Analyzer, background and bands might be indiscernible if it is a monochrome image. In this case, when the image is pseudo colorized, bands become easier to see compared with the monochrome image. Pseudo colorization is also a change on the display only and no change is made in the intensity for measurement, therefore the same measurement results will be obtained regardless of the application of pseudo colorizing. It is also possible to convert and save the pseuo-colorized images an 8bit TIFF, BMP, or JPEG file.



Changing image grayscale (number of bits)

The CS Analyzer is compatible with 8 to 16bit images. These images can be changed to lower bit and higher bit irreversible image mode conversion function. The image file can also be saved after conversion. We recommend saving a separate copy of the original unaltered image.

Changing the image resolution

CS Analyzer can change the resolution of the image to double or half irreversibly changing in the resolution is an effective way to print a smooth image.

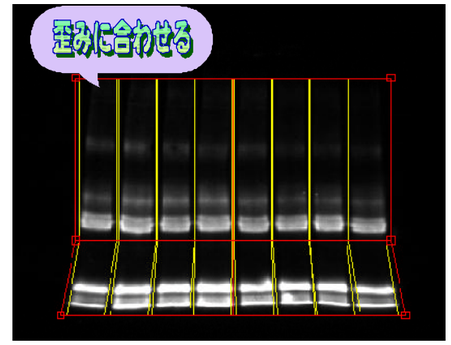
Images with doubled resolution require four times as much space when saved.

Handling a distorted pattern

■ Distortion correction function

Even if the pattern is widened towards the bottom like PAGE, when the distortion correction function is used, the pattern exactly fits the bending of the lane as shown in the figure.

Matching with the distortion



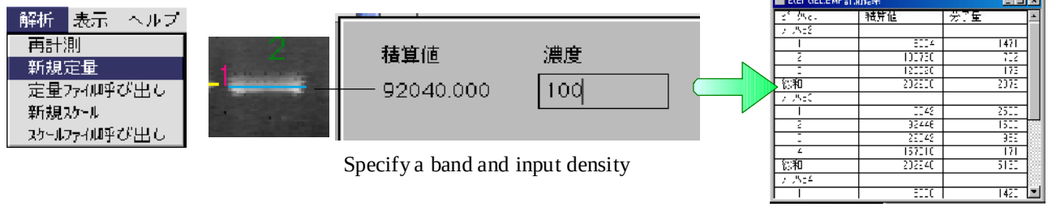
PAGE pattern toward the bottom.

Fixed quantity measurement result

■ New fixed quantity function

Comparative quantitation is available using known density or standard band.

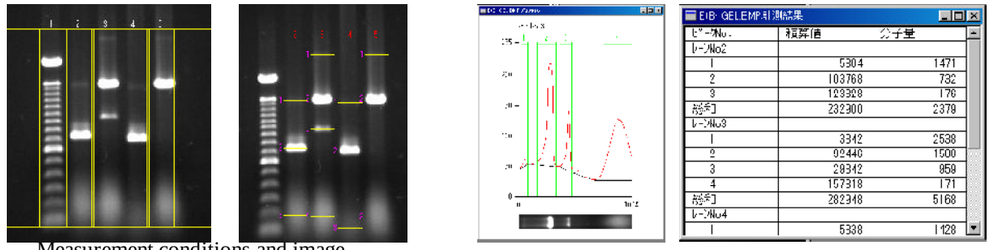
When a certain band is set as "100", the raito of other band is added to the measurement result.



Specify a band and input density

Saving function for analyzed data

The measurement value can be saved together with the image, profile and so on, so it is possible to reanalyze the measurement result under the same condition as before (image conversion, area setting, measurement condition, etc.). Additionally, the profile and measurement results can be individually saved as text data. The text data can be read with spreadsheet software such as Microsoft Excel.



Measurement conditions and image (including image conversion)

Profile

Measurement results

Saved as an analytical result

Saved data is analyzed again

Analytical result (text data) is sent to other software

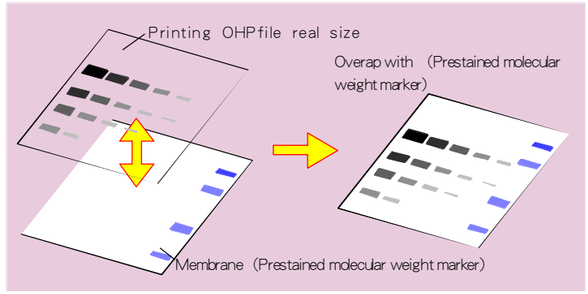
CS Analyzer analytical functions

Molecular size measurement of luminescence pattern

How can we check the position of the bands on a luminescence detected sample that has been subjected to antibody reaction?

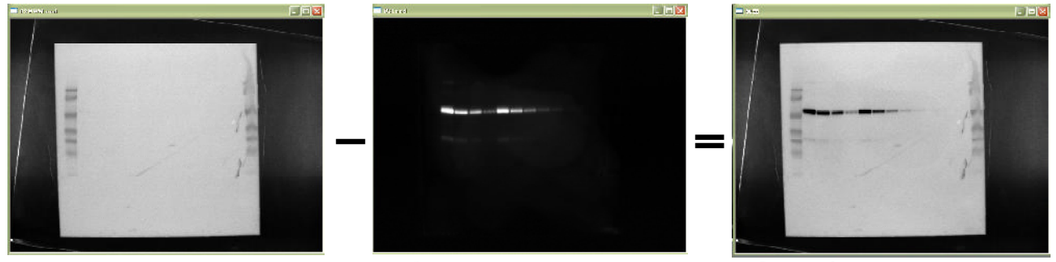
■ Method 1: Use full scale printing

Calibrate the scale of the captured image and print it full size. When it is printed on OHP film, etc., it becomes possible to overlap with membrane and check with a prestained molecular weight marker in the same way as for X-ray film.



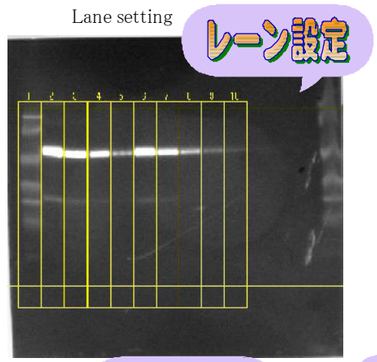
■ Method 2: Measure the molecular size by using image composition.

By composing (subtracting) the image of prestained molecular weight marker and luminance pattern, it is possible to measure molecular-size using analysis software.



Prestained molecular weight marker image — Luminescence pattern — Composed pattern --> To molecular size measurement

Lane setting



● Analyse function

CS Analyzer, the density, molecular-size, etc. of the band can be measured from the electrophoresis pattern.

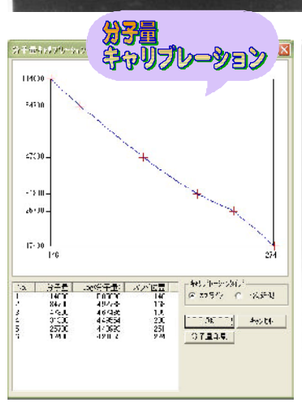
Procedure for molecular-size measurement

-Invert the image

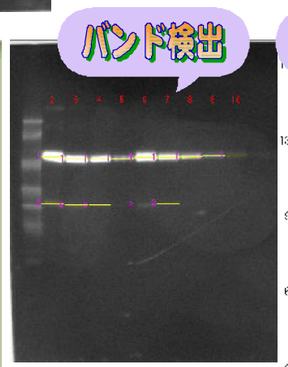
-Set the lane --> Calibration molecular-size.

-Detect band ....measurement result

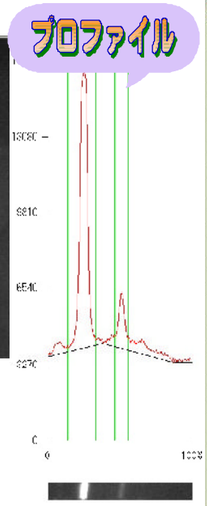
CS Analyzer estimates the molecular-size of the band for the measurement based on the mobility of the molecular-size marker and the molecular-size of each band.



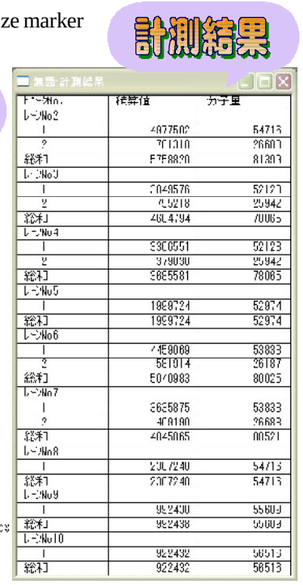
Molecular-size calibration



Band detection



Profile



Measurement result

Grayscale inversion

To convert CBB stained gel into a suitable image for measurement, the image is analyzed after the absorbance is converted. When absorbance conversion is performed, it is possible to convert it into a suitable image for while retaining quantitation. When the absorbance is converted, the image looks as if it is inverted. In addition, the band can be made easier to see by adjusting-contrast. If shading compensation is performed when capturing the image, the background becomes uniform.

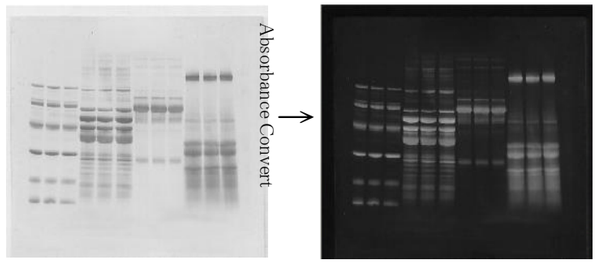
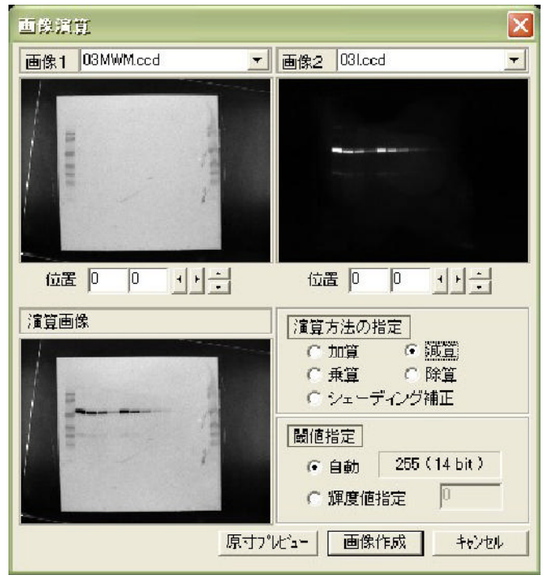


Image calculation functions

■Example 1: Composition of luminescence pattern and colored marker image

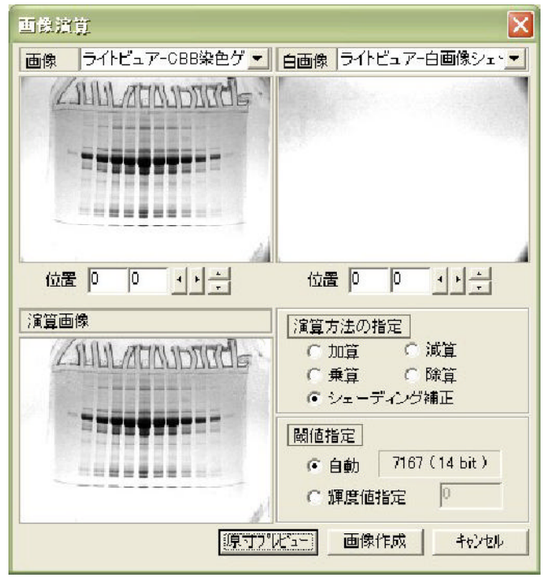
When taking an image of a chemical luminescence membrane, the luminescence pattern can be seen usually but molecular-size markers that don't emit light is are not detected.

However, CS Analyzer (When colored marker is used.) is able to compose (image substraction) the image of a membrane photographed using a white light source and the image of the luminance pattern photographed subsequently. With this image, the molecular-size of chemical luminescence pattern can be measured.



■Example 2 : Shading compensation

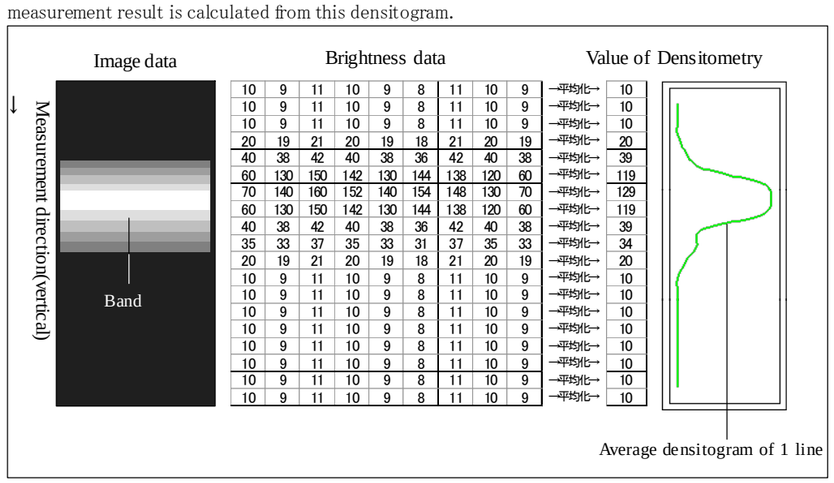
A white transilluminator is used to capture CBB stained gels, etc. This light scource has unevenness, therefore if used as it is; the evenness on the background becomes a problem at analysis. The irregularity of the source of light is corrected when the shading compensation function is used, and the background can be evened out (profer correction might not be possible when the light source has extremely large irregularity.



CS Analyzer analytical functions

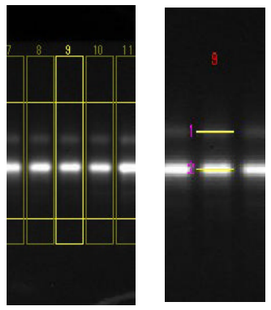
Principle of image analysis(Zone Densitometry method)

At first, set the measurement area (rectangular or square) for the image. Select the measurement direction (vertical, horizontal) when setting the measurement area. Under the zone densitometry method, a densitogram (profile) is obtained using the average value of intensity of pixels for 1 line in the width direction against the measurement direction. The integration value of the measurement result is calculated from this densitogram.



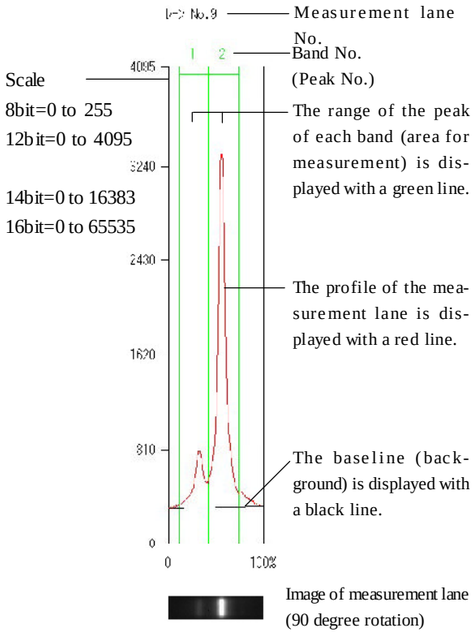
10	9	11	10	9	8	11	10	9	10
10	9	11	10	9	8	11	10	9	10
10	9	11	10	9	8	11	10	9	10
20	19	21	20	19	18	21	20	19	20
40	38	42	40	38	36	42	40	38	39
60	130	150	142	130	144	138	120	60	119
70	140	160	152	140	154	148	130	70	129
60	130	150	142	130	144	138	120	60	119
40	38	42	40	38	36	42	40	38	39
35	33	37	35	33	31	37	35	33	34
20	19	21	20	19	18	21	20	19	20
10	9	11	10	9	8	11	10	9	10
10	9	11	10	9	8	11	10	9	10
10	9	11	10	9	8	11	10	9	10
10	9	11	10	9	8	11	10	9	10
10	9	11	10	9	8	11	10	9	10
10	9	11	10	9	8	11	10	9	10
10	9	11	10	9	8	11	10	9	10

※Note:In an actual measurement, neither the brightness data nor the average value of 1 line can be saved under [Text Save] in the Save Menu.

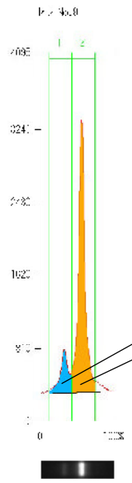


① Lane setting/ Band detection

After the measurement lane is set, the band is detected automatically. The profile for the measurement is displayed as shown in the figure below.



②Display of profile



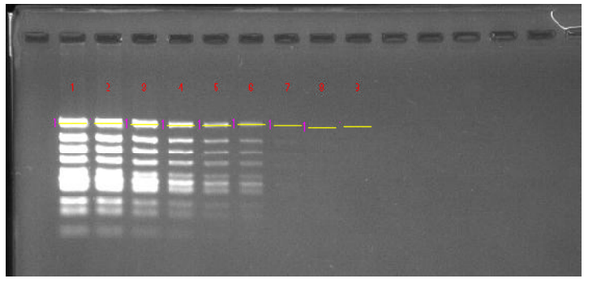
Measurement value

The measurement value of the band is calculated using the integration value of the area enclosed by the profile, the range of the peak, and the baseline (painted part in the Fig. at left). Measurement result is displayed “as an integrated value”.

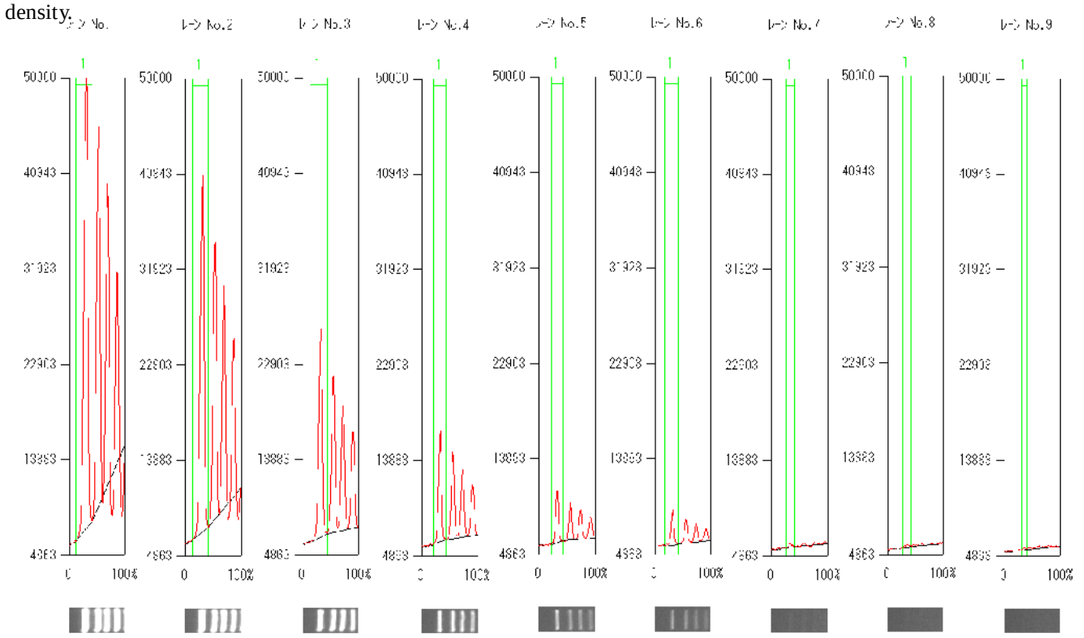
ﾋﾞｰﾝNo.	積算値	ﾊﾟｰｾﾝﾄ
ﾚｰﾝNo9		
1	201152	16.143
2	1044512	83.852
総和	1245664	100.000

Example of analyzing a photographic image and dilution series of DNA

This is the image measured by CS Analyzer after electrophoresis of dilution series of DNA using PrintGraph, photographing ethidium bromide stained gel and saving as TIFF (10bit) file.



Measured profile of each lane is shown below. The density of the band is calculated from the profile as “an integrated value”. Additive amount is total DNA amount of each lane, and the weight of the band of 1057bp becomes approx 20% of the total. Comparing to lane No. 9, lane No.1 has approx 240 times the density.



ATTO0008(10bit).cmd-計測結果

ﾋﾞｰﾝNo.	積算値
ﾚｰﾝNo1	
1	7736550
総和	7736550
ﾚｰﾝNo2	
1	5788470
総和	5788470
ﾚｰﾝNo3	
1	2968110
総和	2968110
ﾚｰﾝNo4	
1	1299000
総和	1299000
ﾚｰﾝNo5	
1	576870
総和	576870
ﾚｰﾝNo6	
1	376110
総和	376110
ﾚｰﾝNo7	
1	47790
総和	47790
ﾚｰﾝNo8	
1	28830
総和	28830
ﾚｰﾝNo9	
1	10530
総和	10530

	Additive 添加量/lane	Target band 対象バンド	Detection 検出	Magnification 倍率
①	1500ng	1057bp	◎	240
②	1000ng	1057bp	◎	160
③	500ng	1057bp	◎	80
④	250ng	1057bp	◎	40
⑤	100ng	1057bp	◎	16
⑥	500ng	1057bp	◎	8
⑦	25ng	1057bp	◎	4
⑧	12.5ng	1057bp	○	2
⑨	6.25ng	1057bp	△	1
⑩	3.13ng	1057bp	×	0.5
⑪	1.61ng	1057bp	×	0.25