FUJIFILM

Application Note

SCIENCE IMAGING SYSTEMS

Application Note No.13

Controlling the contrast of SCIENCE IMAGING SYSTEM images

BAS-5000 / BAS-2500 / BAS-1800II / FLA-3000 / LAS-1000 / LAS-1000plus / FDL5000

BAS

FLA

LAS

FDL

Foreword

How can I get better images? How can I make those faint bands easier to see? These are just a couple of the frequently asked questions regarding image quality. The answer is almost always the same; optimize contrast control. Among the various processing functions affecting image quality, contrast control is the most critical.

SCIENCE IMAGING SYSTEMS (BAS series, FLA series, LAS series and FDL series) produce images that can be digitally processed to control contrast. This contrast control capability adds even more power to the image analysis and image presentation features these systems offer.

Precise image analysis and good image presentation is mostly a matter of understanding digital image fundamentals and knowing how contrast control works in the system you are using. Application Note No. 13 will help you with both.

Contents

Summary

- More gradations and more pixels make a finer image.
- Contrast control - a combination of contrast adjustment and brightness adjustment.
- Each system has its own unique image histogram.

1 Digital image basics

Digital image concept

A digital image is composed of many picture elements (pixels) arranged in rows (X direction) and columns (Y direction). The pixels are usually square and the size of the image is expressed as the number of X-direction pixels times the number of Y-directions pixels (X x Y). Increasing the number of pixels brings out finer details but makes the image larger.

Image density is expressed as gradation level. The density range between white and black is divided into a number of gradation levels suitable for the purpose and each level is assigned a binary level number. Each pixel can therefore be expressed as a level number corresponding to its density. The total number of levels between white and black is called the bit number.

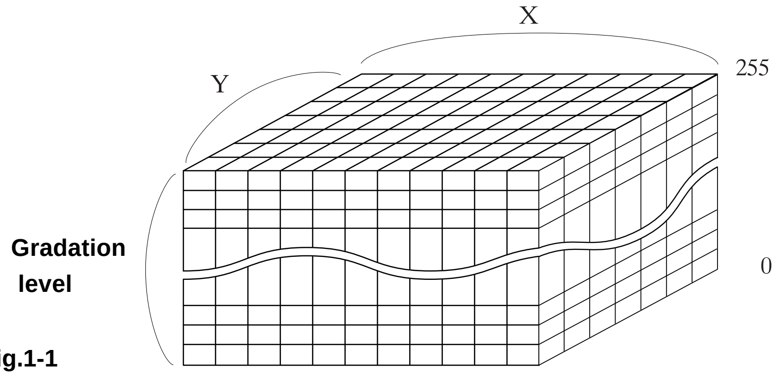


Fig.1-1

Levels vs bit number

Levels	Bit number (Byte number)
256	8 (1)
1024	10 (2)
4096	12 (2)
16384	14 (2)
65536	16 (2)

Image size

Image size (bytes) = Pixels per column x Pixels per row x Byte number

Bit and byte number

Binary System	Decimal System
0	0
11111111	255

Bit number corresponds to the number of figures in binary system. For instance, in binary system, maximum number of 8-bit is " 11111111" which is " 255" in decimal system. So 8-bit has 256 levels from 0 to 255. Byte number corresponds to the memory in computer, and one unit is 8 bits by definition. Image size is effected by byte number.

Fig.1-1 Conceptual view of digital image (256 gradation levels expressed in 8 bits)

Image histogram

A histogram of an image shows how many pixels of each level number makes up the image. The number of pixels is represented on the vertical axis and the gradation level on the horizontal axis.

Contrast control is easier when done with reference to an image histogram displayed beforehand.

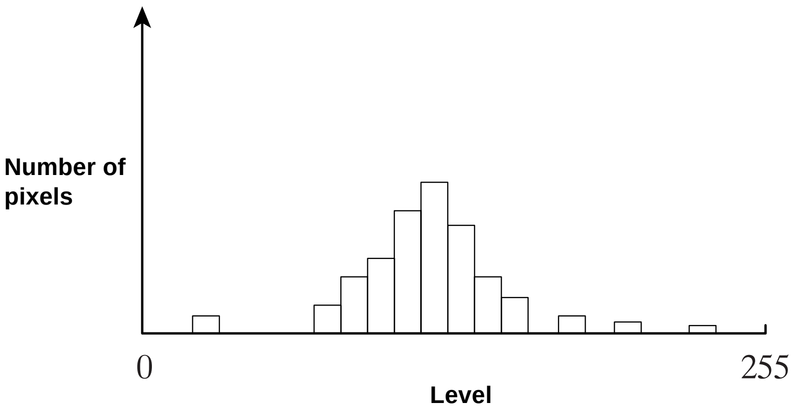


Fig.1-2

Fig.1-2 Example of 8-bit digital image histogram

2 Contrast adjustment using Image Gauge

Contrast control and quantitation

The Image Gauge software carries out contrast control by assigning each image pixel a gradation level corresponding to its gray level. It does not change the values of the original data and therefore has no effect on quantitation.

The procedure involves the following two steps.

First step (Range Scope function)

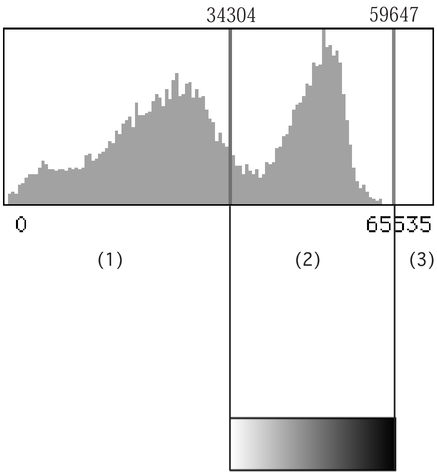


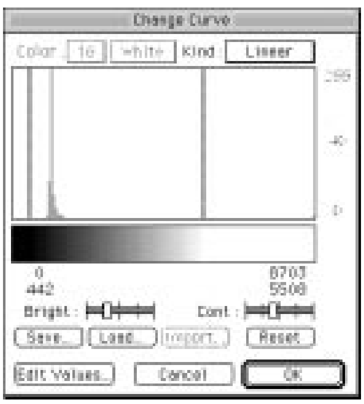
Fig. 2-1

Fig.2-1 Range Scope function

The gray level range is roughly defined by adjusting the Low/High adjuster bars based on the histogram. In Fig.2-1, range (1) is assigned to white, range (2) to a gradation between white and black, and range (3) to black. In this example, gray level values between 34304 and 59647 are assigned to gradation levels between white and black. As most of the image noise is on the left side, this operation decreases the noise factor.

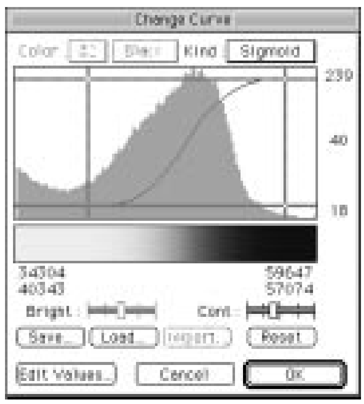
Second step (Brightness/Contrast function)

The gray levels within the limited range defined in the first step are converted to gradation levels using a conversion curve. Five conversion curves are available: Linear, Sigmoid, Curve, Triangular and Free Hand. You can fine tune the low/high limit and adjust shape of the conversion curve by dragging the cursor in the change curve window. This process can produce a sharp image with distinct black and white regions.



Linear

Gray levels are linearly converted to gradation levels. Linear conversion is a good choice when the system generates data concentrated within a narrow range.



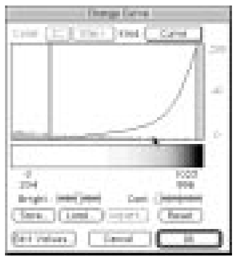
Sigmoid

Sigmoid conversion renders intermediate densities with high clarity.

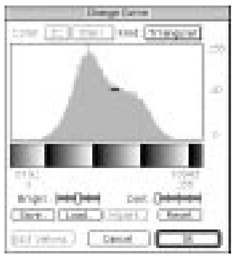
Fig. 2-2

Fig.2-2 Brightness/Contrast function

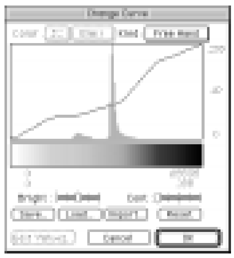
Other conversion curves are shown below.



Curve



Triangular



Free hand

3 Application to different system images

BAS system image

A BAS image histogram often includes a broad background peak and a narrow sample data peak. The background includes natural noise picked up owing to the high sensitivity of the imaging plate (IP). Reducing background noise by contrast control, contributes to make clear image. The enhancing effect of contrast control is explained in the case of a whole-body autoradiography image such as taken up in the following explanation.

BAS System

This is an IP reader system.
The system is designed to have high sensitivity and high resolution.
The system is used in the field of autoradiography, X-ray crystallography, etc. The BAS-1800II can read 23 x 25cm IP and BAS-2500 can read 20 x 40cm IP at 50 micron pixel size. BAS-5000 has special confocal optics and can read 20 x 25cm IP at 25 micron pixel size.

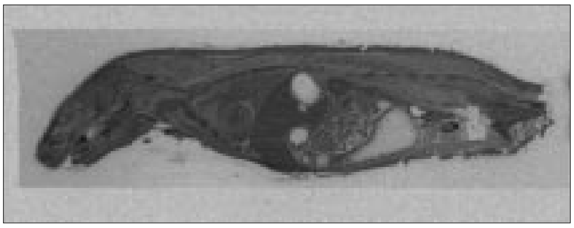


Fig.3-1-a

In the histogram of Fig.3-1-b, Low/High adjuster bars are set to enclose the peak on the right. This gives the image shown in Fig.3-1-c.

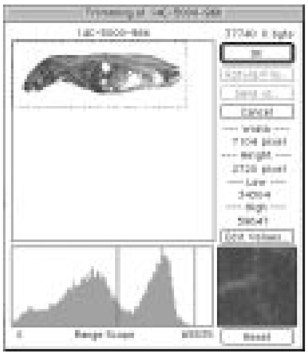


Fig.3-1-b

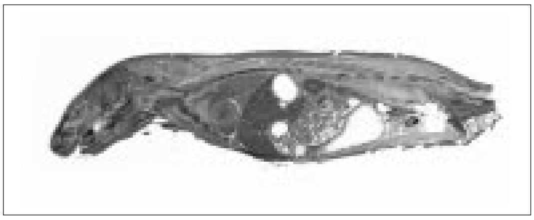


Fig.3-1-c

Fig.3-1 Image taken by BAS

a (upper) : Original image
b (middle left) : Range Scope window
c (middle right) : Image processed by Range Scope function
d (lower left) : Change Curve window with Sigmoid selected
e (lower right) : Image after Sigmoid conversion

The image of Fig.3-1-c has low background but the dark internal organ part can be improved. Conversion is therefore carried out using the Sigmoid curve shown in Fig.3-1-d. The result is a clear and low-background image shown in Fig.3-1-e.

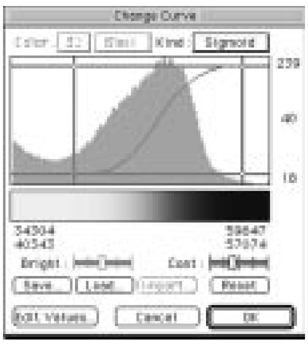


Fig.3-1-d

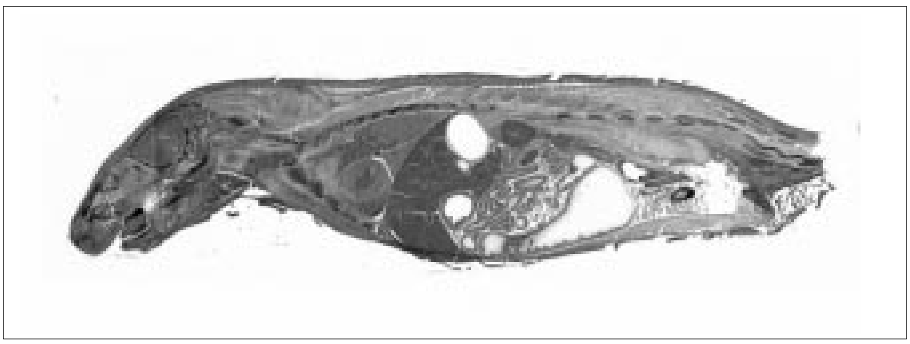


Fig.3-1-e

The relation between exposure time and BAS image

Although BAS can detect a signal with very short exposure time, a characteristic of BAS is that imaging of details improves with longer exposure time. This can be seen from the following images and their partial enlargements, taken with different exposure time and subjected to only slight contrast control.

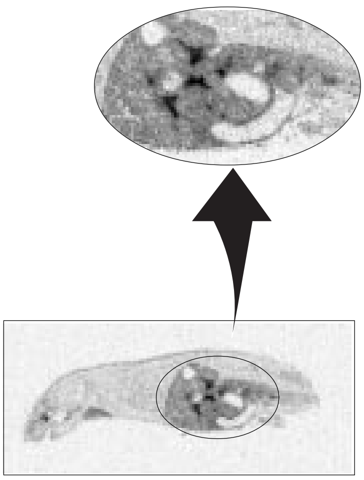


Fig.3-2-a

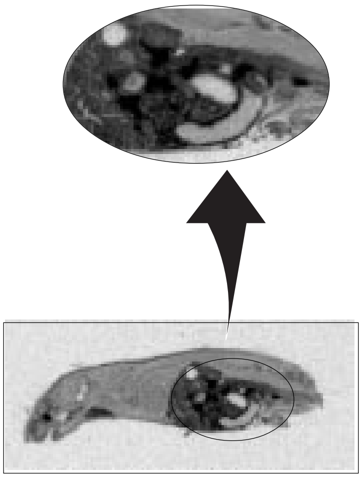


Fig.3-2-b

Fig.3-2 Effect of exposure time
a (left) : 1 hour exposure
b (right) : 24 hours exposure
Read with BAS-2500

Positive and negative image expression

The gradation of an image can be reversed. This function is useful for luminescent image of LAS system. In the original LAS system image (Fig.3-3-a), the luminescent part appears as white. The reversed image (Fig.3-3-b) looks like what is obtained when film is exposed to luminescence.

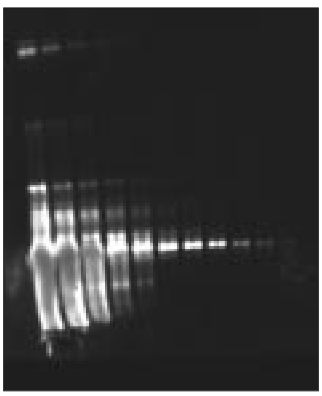


Fig.3-3-a

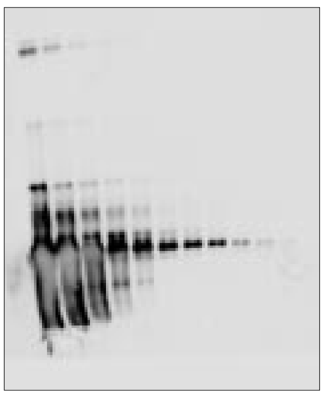


Fig.3-3-b

Fig. 3-3 Positive/negative image reversal
a (left) : Positive image
b (right) : Negative image
Taken with LAS-1000plus

LAS system chemiluminescent image

Chemiluminescence produces only an extremely weak image signal, so that the LAS chemiluminescent image consists mainly of dark background. The luminescent data tends to concentrate at a narrow region of the histogram. Application of contrast control to an LAS chemiluminescent image therefore produces a dramatic effect.

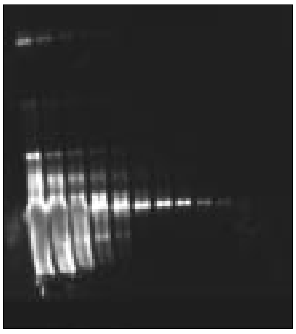


Fig.3-4-a

In the histogram of Fig.3-4-b, Low/High adjuster bars are set to enclose the high data density region. This gives an image with sharply defined contrast (Fig.3-4-c). This Range Scope function is automatically performed by the LAS system when reading the image. This means that the images of Fig.3-4-a and Fig.3-4-c are the same.

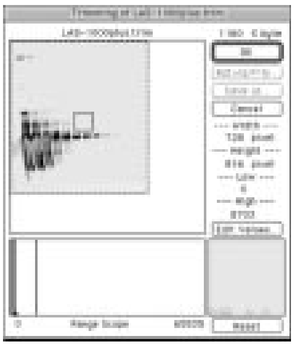


Fig.3-4-b

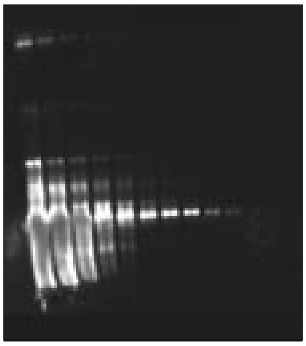


Fig.3-4-c

Linear conversion is carried out next (Fig.3-4-d). This narrowing of the low/high limit enables detection of low density portions as shown in Fig.3-4-e.

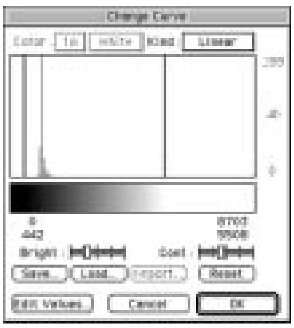


Fig.3-4-d

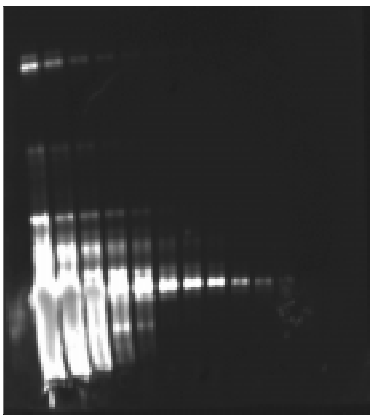


Fig.3-4-e

LAS System

This system consists of a highly sensitive cooled CCD camera which has 1.3 million pixels and a special f0.85 lens. The high sensitivity comes from the large size (11 microns) square pixels, each with its own individual microlens, and cooling to -30˚C. Also the exposure time can be set up to one hour by the Image Reader software and can expose longer by the bulb function.

Bulb function

In bulb function, image capturing will continue until clicking the “stop” button. This function is useful in case of overnight exposure.

Fig.3-4 Image taken by LAS

a (upper) : Original image
b (middle left) : Range Scope window
c (middle right) : Image processed by Range Scope function
d (lower left) : Change Curve window with Linear selected
e (lower right) : Image after Linear conversion

FLA system fluorescent image

The histogram of an FLA fluorescent image has two high data density regions. The one on the left side is characterized by a large background component.

Fig. 3-5-a

In the histogram of Fig.3-5-b, Low/High adjuster bars are set to enclose the peak on the right. This gives an image with low background (Fig.3-5-c)

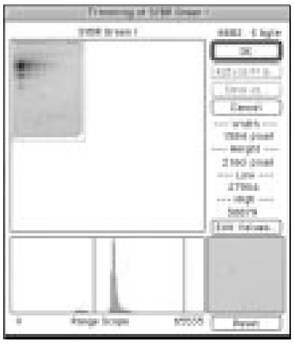


Fig.3-5-b

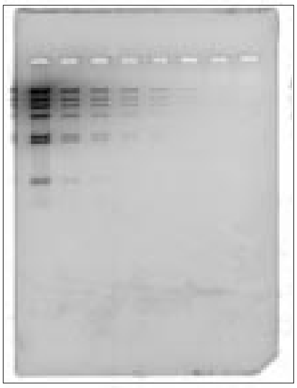


Fig.3-5-c

Linear conversion is then carried out to heighten the contrast (Fig.3-5-d). This makes faint bands distinctly discernible (Fig.3-5-e).

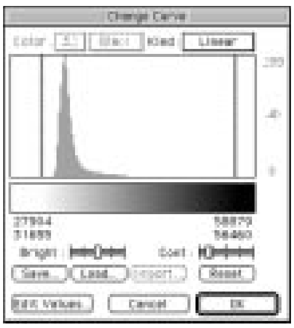


Fig.3-5-d

Fig.3-5-e

FLA System

This fluorescent scanning system can be equipped with up to three lasers; a 473 nm SHG* solid state laser, a 532 nm SHG* solid state laser and a 633nm He-Ne laser. High speed scanning can read a 23 x 40cm IP at 50 micron pixel size in 6 minutes; generating a 70MB image data file. The system can read not only IP but also glass plates for DD applications and 96-well plates.

*SHG
Secondary Harmonic Generation.
A new type of laser produced by Fuji Photo Film.

Fig.3-5 Image taken by FLA

a (upper) : Original image
b (middle left) : Range Scope window
c (middle right) : Image processed by Range Scope function
d (lower left) : Change Curve window with Linear selected
e (lower right) : Image after Linear conversion

FDL system image

The properties of an FDL image differ depending on whether it is an optical image or a diffraction image. The histogram of an FDL diffraction image has broad gradation level distribution. This makes contrast control unnecessary. An FDL transmission image has a histogram resembling that of a BAS image and can be markedly enhanced by contrast control.

(Transmission image)

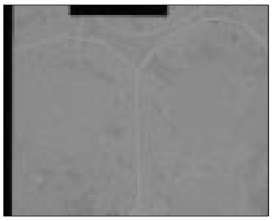


Fig.3-6-a

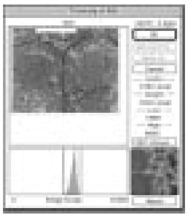


Fig.3-6-b

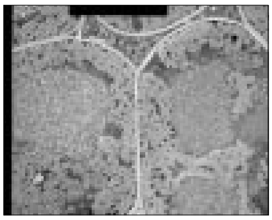


Fig.3-6-c

In the histogram of Fig.3-6-b, Low/High adjuster bars are set to enclose the high data density region. This produces a sharp image (Fig.3-6-c).

Linear or Sigmoid conversion is generally used with an FDL image. The choice is made based on image appearance. Linear conversion is suitable for the image shown here (Fig.3-6-d). This narrowing of the low/high limit gives an image with sharply defined contrast (Fig.3-6-e).

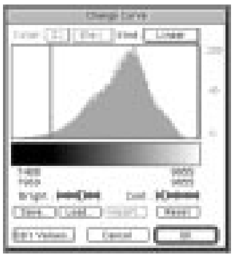


Fig.3-6-d

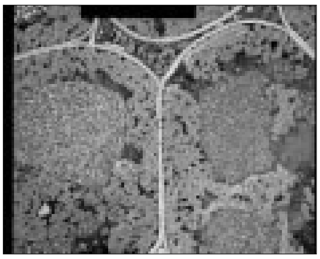


Fig.3-6-e

FDL System
This system is designed for TEM image detection.
The cassette accepts up to 32 IPs of 99.6 mm x 80.9mm specially designed to fit the film stage of electron microscopes. Image can be scanned at 25 micron pixel size.

Diffraction
Diffraction image of crystals

Fig.3-6 Image taken by FDL

a (upper) : Original image
b (middle left) : Range Scope window
c (middle right) : Image processed by Range Scope function
d (lower left) : Change Curve window with Linear selected
e (lower right) : Image after Linear conversion

4 References

1) Ed. by Donat-P. Häder., Image analysis in biology, CRC Press (1992)
2) Glasbey, C.A., Horgan, G.W., Image analysis for the biological sciences, John Wiley & Sons (1995)
3) Grotta D. Grotta S. W., The illustrated digital imaging dictionary, McGraw-Hill (1998)

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