TECHNICKÁ UNIVERZITA V LIBERCI

Nové možnosti rozvoje vzdělávání na Technické univerzitě v Liberci

Specifický cíl A2: Rozvoj v oblasti distanční výuky, online výuky a blended learning

NPO_TUL_MSMT-16598/2022

Experimental analysis of structures - internal standards

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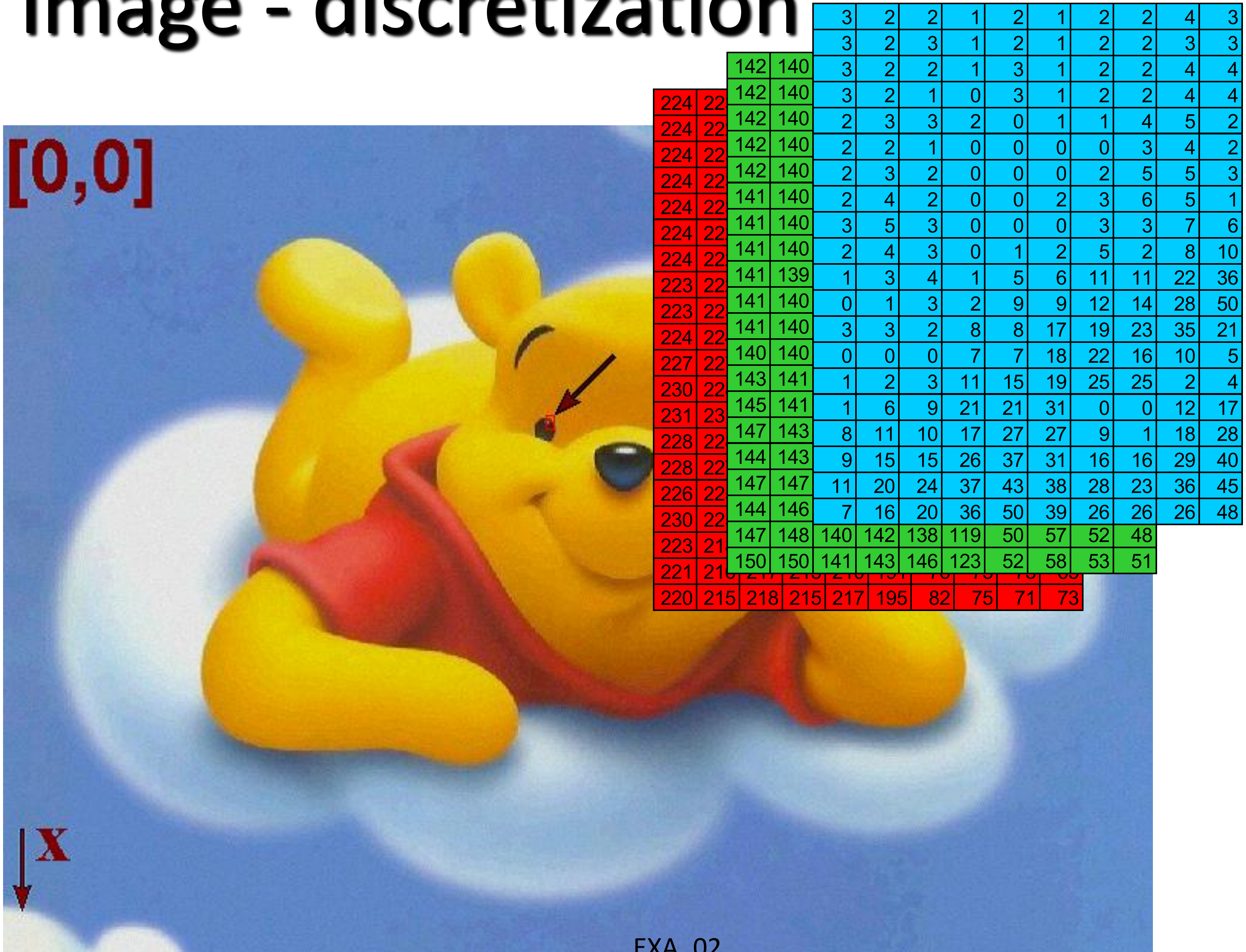
Financováno Evropskou unií NextGenerationEU

Národní plán obnovy

MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY

Image - discretization



3	2	2	1	2	1	2	2	4	3
3	2	3	1	2	1	2	2	3	3
3	2	2	1	3	1	2	2	4	4
3	2	1	0	3	1	2	2	4	4
2	3	3	2	0	1	1	4	5	2
2	2	1	0	0	0	0	3	4	2
2	3	2	0	0	0	2	5	5	3
2	4	2	0	0	2	3	6	5	1
3	5	3	0	0	0	3	3	7	6
2	4	3	0	1	2	5	2	8	10
1	3	4	1	5	6	11	11	22	36
0	1	3	2	9	9	12	14	28	50
3	3	2	8	8	17	19	23	35	21
0	0	0	7	7	18	22	16	10	5
1	2	3	11	15	19	25	25	2	4
1	6	9	21	21	31	0	0	12	17
8	11	10	17	27	27	9	1	18	28
9	15	15	26	37	31	16	16	29	40
11	20	24	37	43	38	28	23	36	45
7	16	20	36	50	39	26	26	26	48

What is an image?

What is an image? An image is a row or matrix of square pixels (picture elements) arranged in columns and rows.

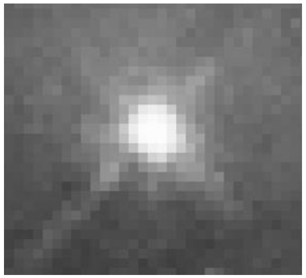


Fig. 1: Image - a row or matrix of pixels arranged in columns and rows.

- In the (8-bit) grey image, each pixel is assigned one value between 0 and 255. A grayscale image is usually called a black and white image, which is incorrect because a grayscale image contains a range of degrees in addition to black and white.

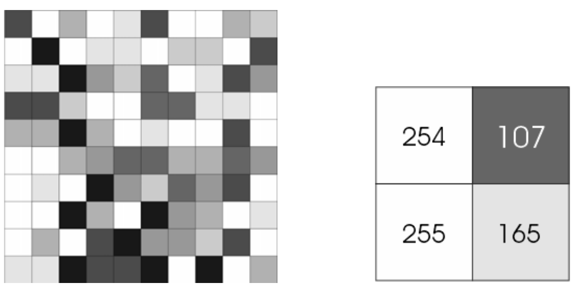


Fig. 2: Each pixel has a value in the interval from 0 (black) to 255 (white). The possible range of pixel values depends on the colour depth of the image, here 8 bits = 256 colour shades or greyscale.

A normal grayscale image has 8 bit color depth = 256 grayscale. A "true colour" image has 24 bit colour depth = 8 x 8 x 8 bits = 256 x 256 x 256 colours = ~16 million colours.

What is an image?

- Some grayscale images have multiple grayscales, e.g. 16 bit = 65536 grayscale.

- **Colour depths used**
- 1-bit color (21 = 2 colors) also referred to as Mono Color (most commonly used is that bit 0 = black and bit 1 = white)
- 4-bit color (24 = 16 colors)
- 8-bit color (28 = 256 colors)
- 15-bit color (215 = 32,768 colors) also referred to as Low Color
- 16-bit color (216 = 65,536 colors) also referred to as High Color
- 24-bit color (224 = 16,777,216 colors) also referred to as True Color
- 32-bit color (232 = 4,294,967,296 colors) also referred to as Super True Color
- 48-bit color (248 = 281,474,976,710,656 = 281.5 trillion colors) also referred to as Deep Color

Image - Resolution

Accepted image types and formats

- The colour image consists of three components representing the intensity of the red, green and blue components. The pixel values for each component range from 0 to 255. Use, for example, to measure the intensity or hue of an image. The grey images are derived images. The pixel values vary from 0 to 255, but are the same for all three components in each pixel.
- Gray images are not inherent to the NIS Elements system. They are created by several transformations, for example by separating components from the RGB representation.
- Binary images have two possible values, 0 for the background and a maximum value of 1 for objects and structures. They are products of segmentation functions such as Threshold and are often referred to as segmented images. They are used to measure shape and size.
- *.bmp, *.jpg, *.tiff,*.jpg2000 (*.jp2), (*.lim)

Accepted image types and formats

NIS-Elements AR™ supports the following standard image formats. In addition, NIS-Elements AR™ uses custom file formats to serve specific image analysis requirements.

JPEG2000 (JP2)

An advanced format with selectable compression ratios. Calibration, vector plane, text labels, and other metadata are saved with the image in this format.

ND2 (ND2)

A special format for storing entire image sequences that are created during ND experiments. It stores a lot of additional information about the equipment used, the experimental conditions and the setup.

Joint Photo Expert Group Format (JFF, JPG, JTF)

Standard JPEG files (JPEG File Interchange Format, Progressive JPEG, JPEG Tagged Interchange Format) are used in many image processing applications.

Tagged Image File Format (TIFF)

This format stores the same amount of meta-data as JPEG2000. TIFF files are larger than JPEG2000, but load faster. There are several versions of the TIFF format that correspond to different ways of storing image data. NIS-Elements AR™ supports common types of this format.

CompuServe Graphic Interchange Format (GIF)

This format uses lossless compression and an 8-bit color scheme. The gif format supports monochrome transparency and animation. GIF does not support layers or alpha channels.

Portable Network Graphics Format (PNG)

This is a format with lossless compression (no LZW) and alpha channel support (different transparency intensities). NIS-Elements AR™ does not support interlaced or transparency versions of PNG files.

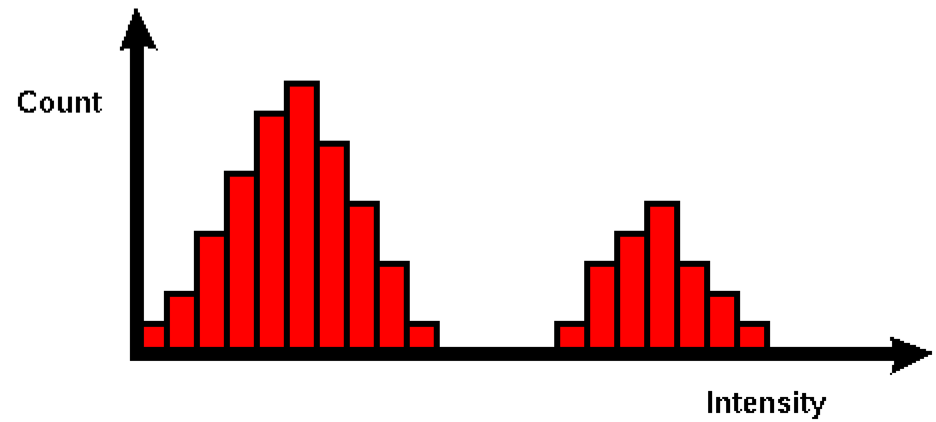
Windows Bitmap (BMP)

This standard Windows format cannot store any additional information (such as author name, subject, calibration, etc.).

LIM Format (LIM)

This format was developed in the past to meet the needs of image analysis systems. Currently, all the features of this format are included in the more modern alternative JPEG 2000.

Image histogram



- In the context of image processing, the histogram tells about the distribution of pixel intensity values in the image.
- For an 8-bit greyscale image there are 256 possible intensity levels, i.e. the x-coordinate will contain the values 0 - 255 and the y-coordinate the number of pixels or their relative frequency.
- Color image histograms - plot individual histograms for the distribution of red, green and blue intensities in an image, or create a 3D histogram.

Image histogram [2]

- Examples of histograms of a dark image, a bright image, a low-contrast image, and a high-contrast image are shown in Figure 3.16.

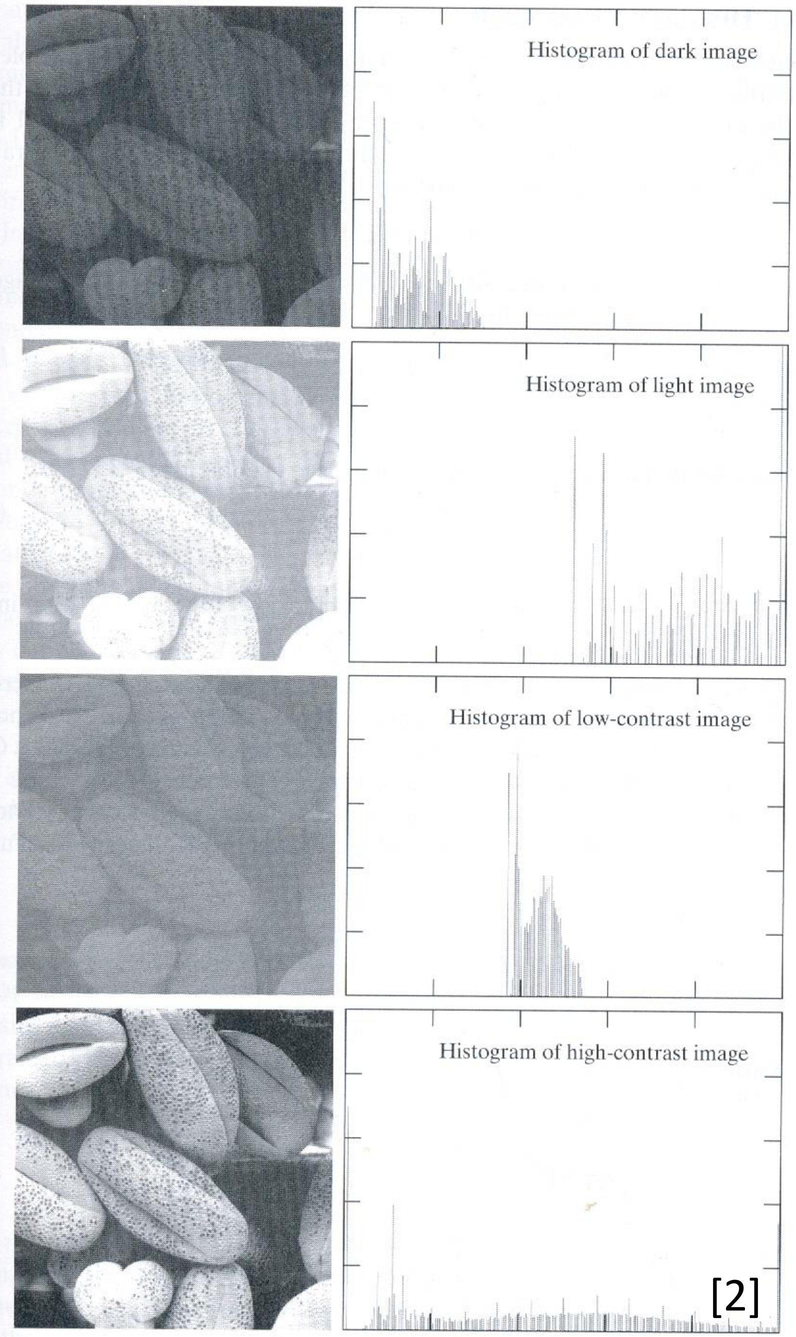
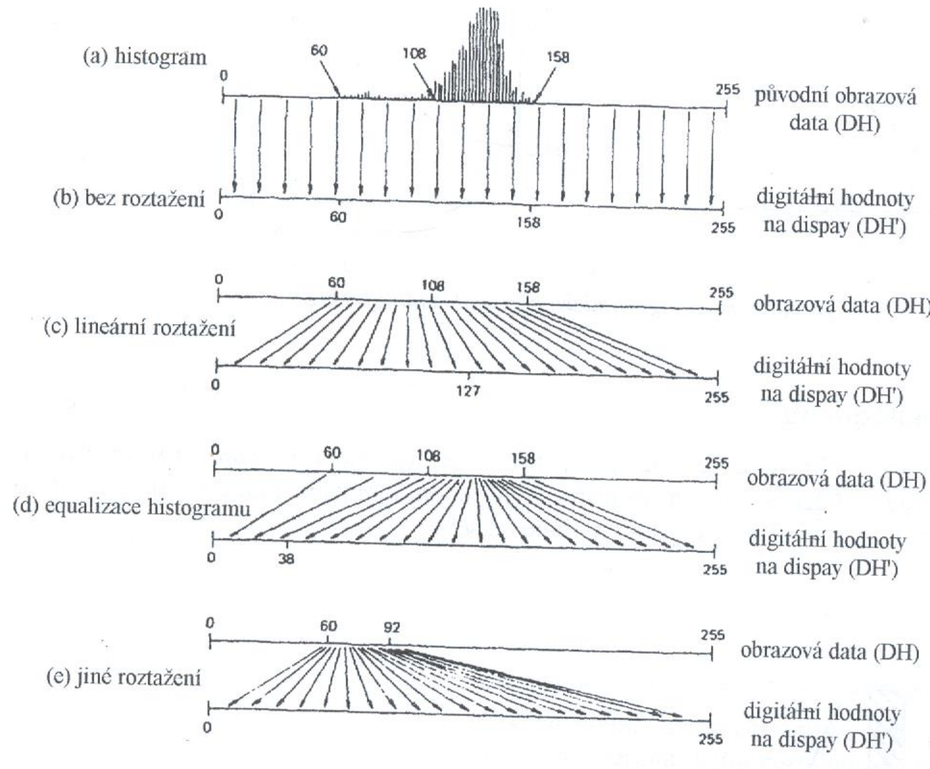


FIGURE 3.16 Four basic image types: dark, light, low contrast, high contrast, and their corresponding histograms.

EXA_02

Image enhancement - histogram stretching [3]

The original digital image usually does not contain pixels whose DH would take all the possibilities given by the encoding (i.e., e.g., values 0-255 in the case of 8-bit data). Their relative representation in the image is shown in the histogram. With normal 8-bit encoding, it happens that the image contains pixels with values using only a certain part of the whole range. If the values are concentrated, for example, in the first third of the possible range, i.e. close to 0 (=black) to 255 (=white), the resulting image will contain only dark pixels with small differences in density and the information will be hard for us to read. There are simple ways to make the information visible by using a grayscale transformation (Figure 31):



Obr. 31. Úprava histogramu [3]

Image enhancement - histogram stretching [3]

1. Linear histogram expansion
2. General histogram modification - it can be a histogram stretching e.g. according to square root, exponential, logarithmic function.
3. Histogram equalization - here the distance between the newly assigned values depends on the frequency of the given values. DHs with low frequencies are closer and those with high frequencies are further apart, see Fig. 1.16 [1] (the figure shows both the histogram of the image intensity and the cumulative frequency).

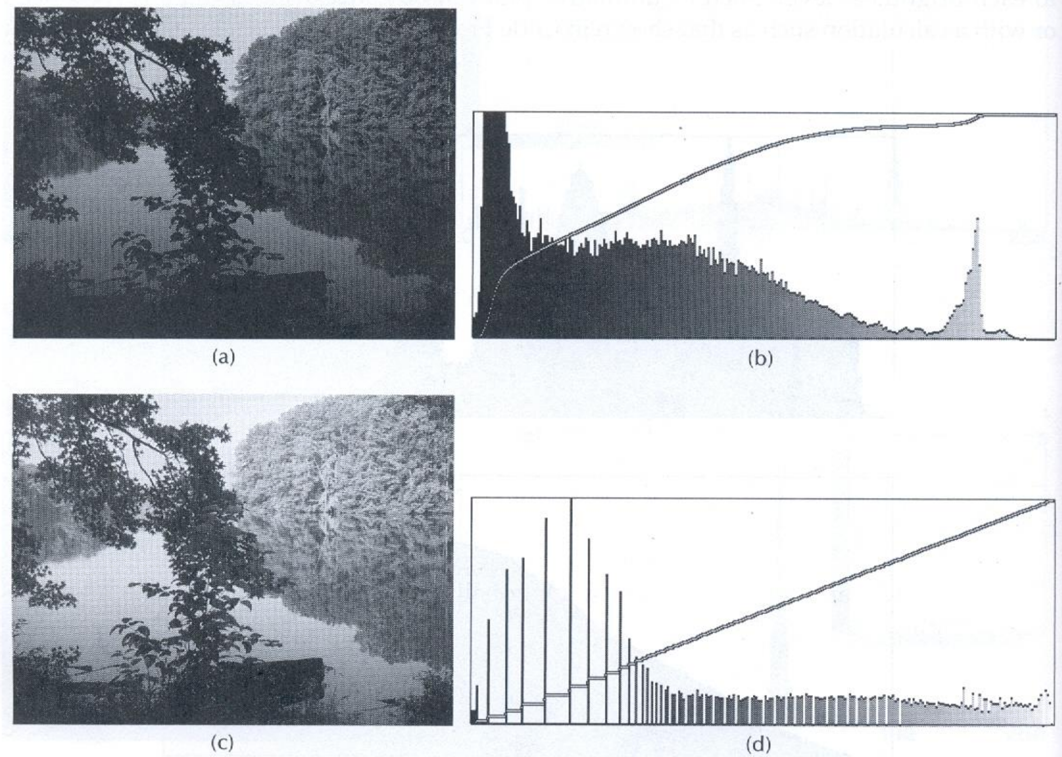


FIGURE 1.16
Histogram equalization: (a) original image [lake.tif], (b) superimposed histogram and cumulative histogram of (a), (c) resulting image after processing, (d) superimposed histogram and cumulative histogram of (c).

[1]

Histogram (highlight) - explanation of the gamma parameter [1]

Non-linear histogram highlighting (stretching)

- The most widely used methods that mimic a photographic darkroom :o) are described by a simple constant - gamma. Photographic film responds logarithmically to light intensity in the same way as human vision. The relationship between film density and light intensity on a logarithmic scale is linear in the middle, see Figure 1.13. the slope of this linear region is determined by the value of gamma. A film with a high gamma value produces images with high contrast, while low gamma values cause a wider brightness interval.
- Example of intensity change based on gamma value change, Fig. 1.14 according to the relation:

$$I = 255\left(\frac{Originál - Minimum}{Maximum - Minimum}\right)^{\gamma}$$

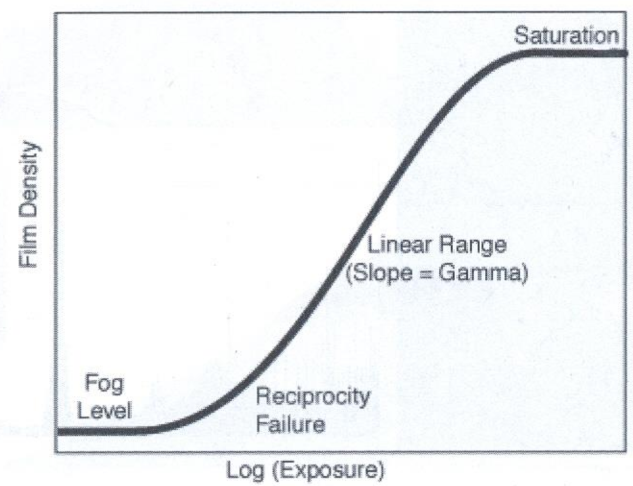


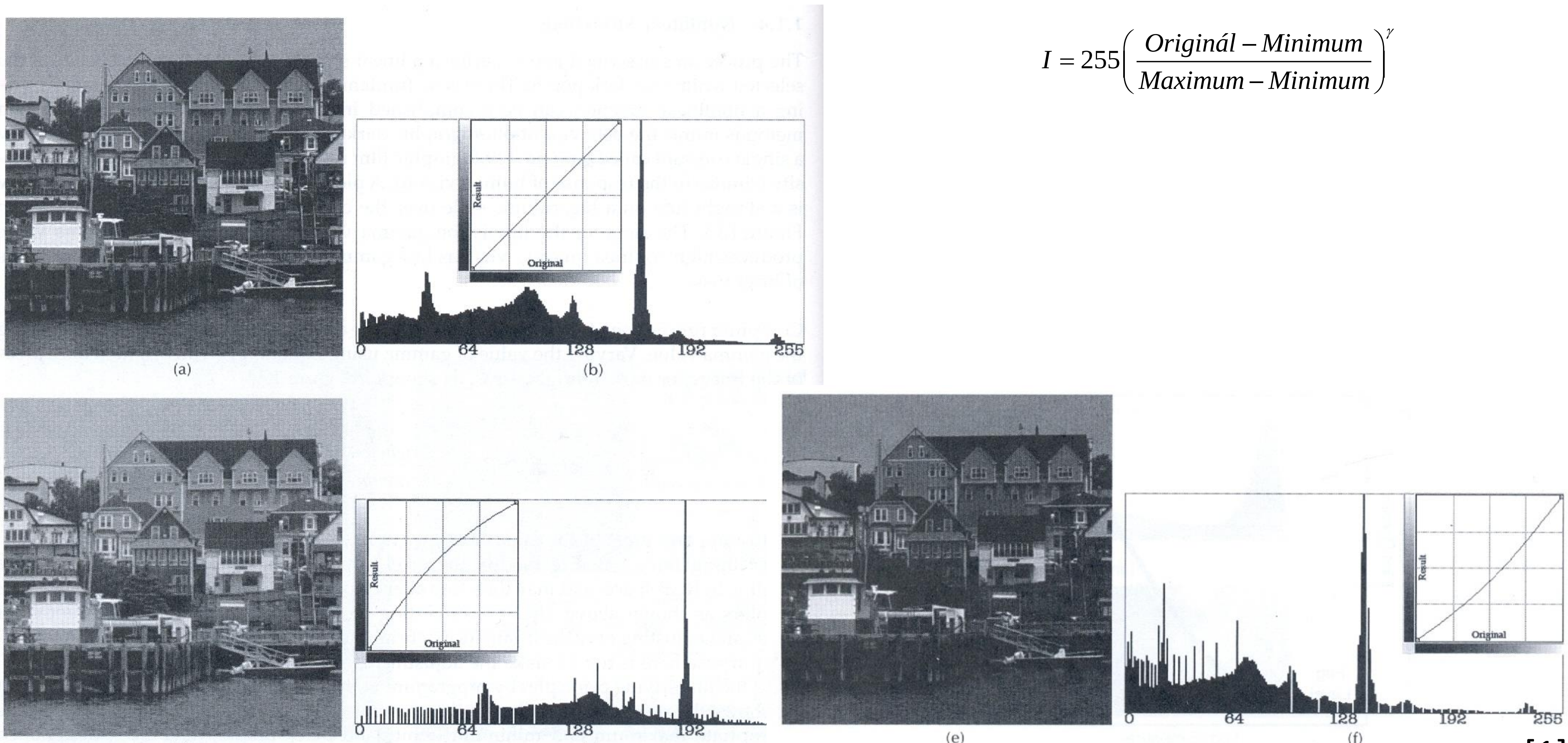
FIGURE 1.13
Diagram of film response to light intensity. High-contrast film has a steep slope in the central linear section, as compared to low-contrast film, which can cover a greater exposure range.

[1]

Histogram (highlight) - explanation of the gamma parameter [1]

- Example of intensity change based on gamma value change, Fig. 1.14 according to the relation:

$$I = 255\left(\frac{Originál - Minimum}{Maximum - Minimum}\right)^{\gamma}$$



[1]

EX **FIGURE 1.14**
Varying gamma: (a,b) original image [harbor.tif] and its histogram, (c,d) adjusting gamma greater than 1 with transfer function and resulting histogram, (e,f) adjusting gamma less than 1 with transfer function and resulting histogram.

Image - resizing [1]

- **The most common -** selecting every second pixel vertically and horizontally (50% of the size), every third pixel (33% of the size), every fourth pixel (25% of the size), ...However, it can hide small details. (When enlarging an image, the operation is reversed, i.e. doubling, tripling, ... a given pixel).
- **Interpolation** - most used for selecting new pixel values.
  - **Bilinear** - uses 4 original pixels that surround a new pixel obtained by linear interpolation from the distances between the original pixels, see Figure 1.27c)
  - **Bicubic interpolation** - uses additional adjacent pixels to create a higher order fitting function, see Figure 1.27d). One of the most widely used methodologies, it produces images with less blurred edges and detail than bilinear interpolation.

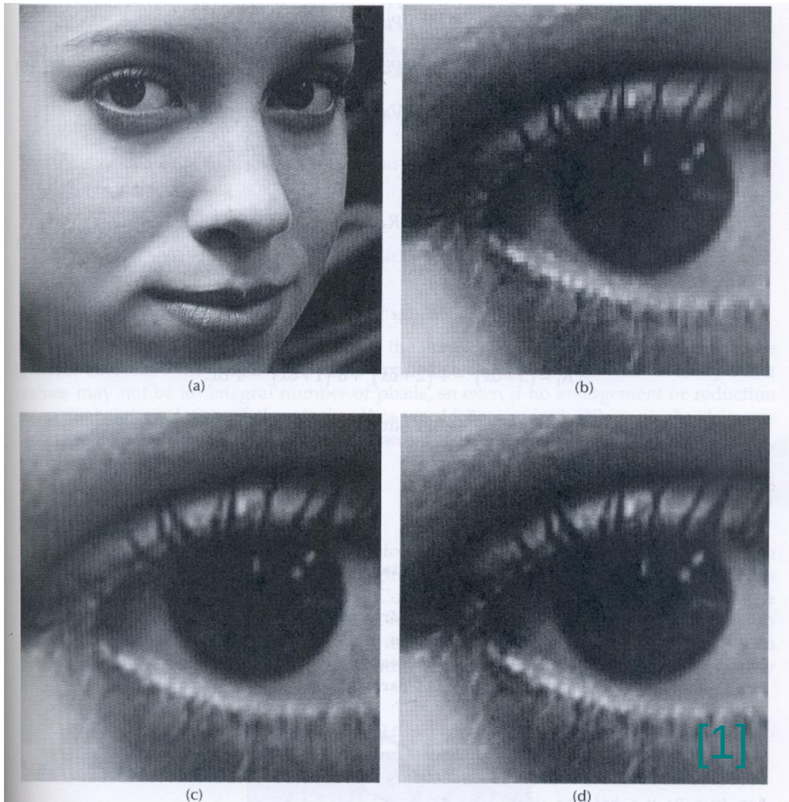
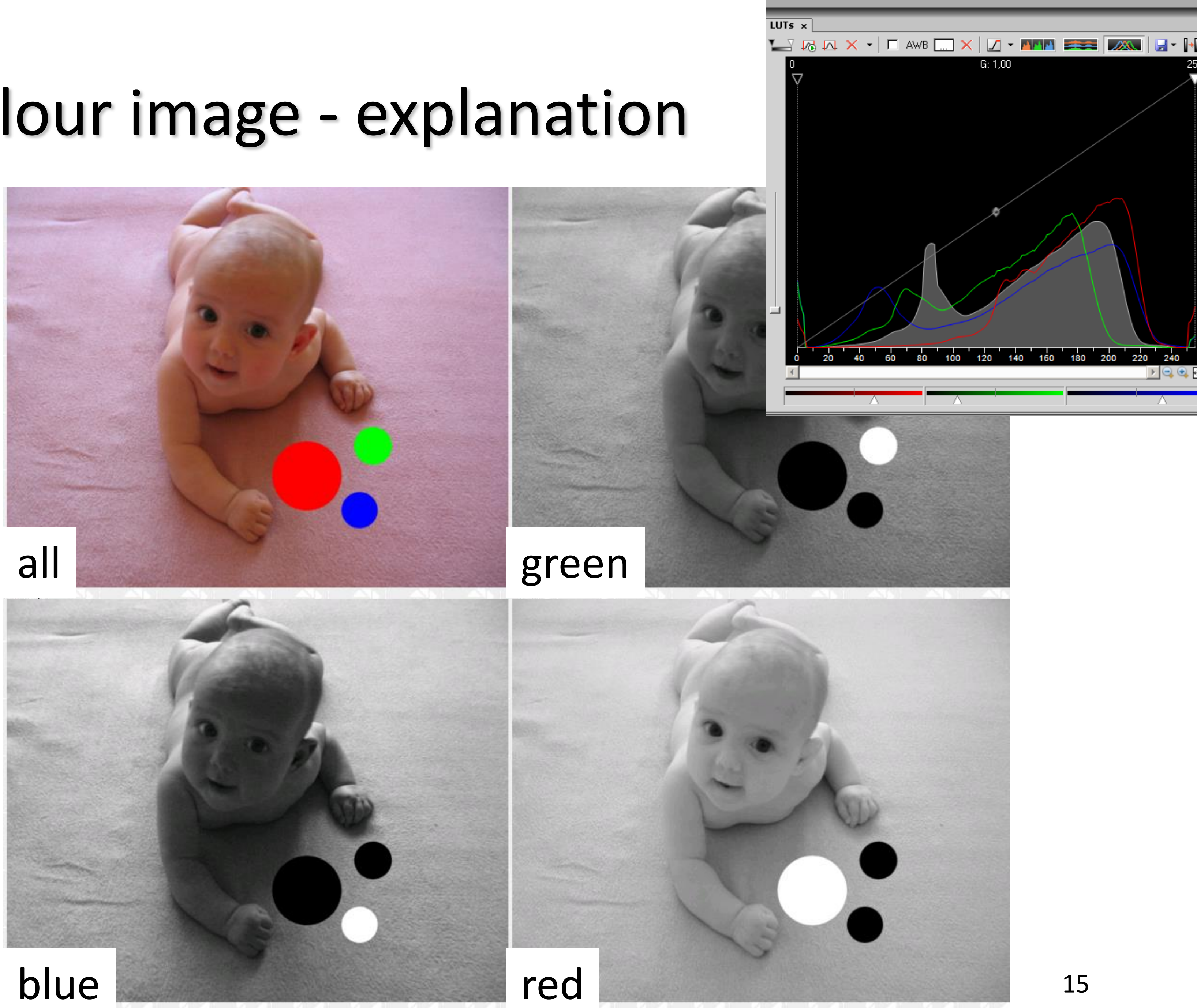
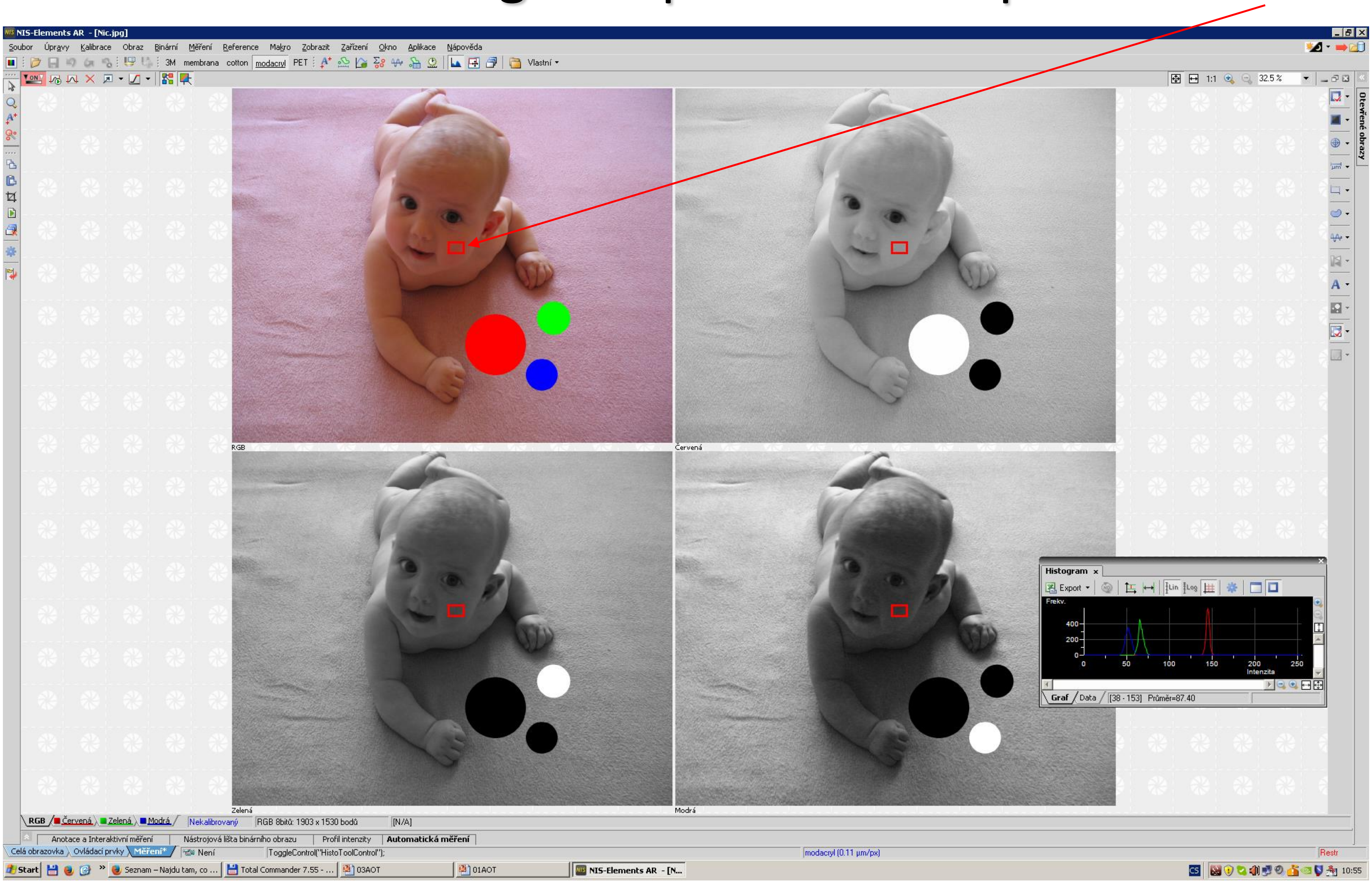


FIGURE 1.27
Enlargement of an image (a) original image [face.tif], (b) detail enlarged 266% by nearest-neighbor method, (c) bilinear interpolation, (d) bicubic interpolation.

Colour image - explanation



Colour image - explanation via probe



Colour image [2]

- The interpretation of colours by the human brain is a physiological-psychological phenomenon that is not fully explained. The physical basis of colours can be expressed on a formal basis supported by experimental and theoretical results.
- 1666 Sir Isaac Newton - a beam of light passing through a glass prism is not white when exiting the prism, but decays into a spectrum of colours from violet at one end of the spectrum to red at the other end, see Figure 6.1.
- If we look at the visible colour spectrum, Figure 6.2, the colours are not strictly separated, but one overlaps the other. The wavelength of the visible colour spectrum is approximately 380 - 780nm.

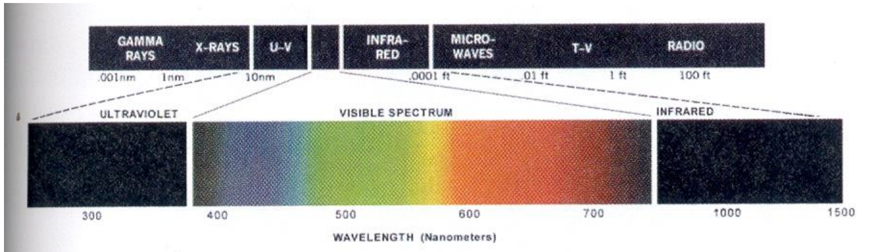


FIGURE 6.2 Wavelengths comprising the visible range of the electromagnetic spectrum. (Courtesy of the General Electric Co., Lamp Business Division.)

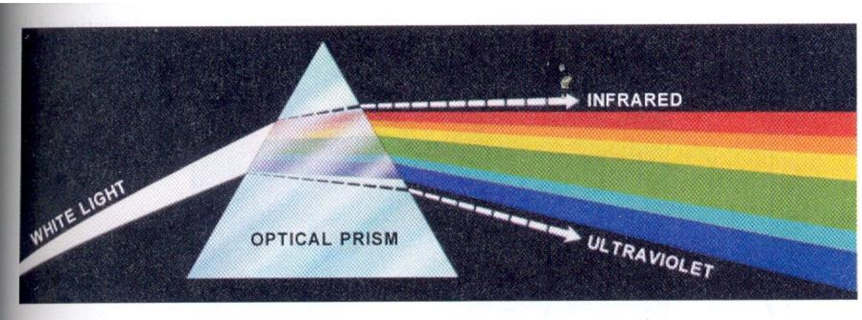


FIGURE 6.1 Color spectrum seen by passing white light through a prism. (Courtesy of the General Electric Co., Lamp Business Division.)

[2]

Colour image

- There are two main colour spaces used in science communication - **RGB** and **CMYK**.
- **RGB - this model is close to human vision.** RGB uses additive color mixing. It is a basic color model that uses TV or other media that project color through light. It is a basic model that is used in PC and web graphics but cannot be used in printing.
- Derived colours from RGB - **cyan, magenta and yellow** - are created by mixing two primary colours (red, green or blue) to the exclusion of a third colour. Red combines with green to create yellow, green and blue to create cyan, and blue and red to create magenta. The combination of red, green and blue at full intensity produces white, see Figure 4 below.

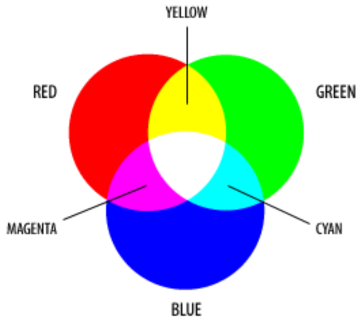


Fig. 4: Additive RGB model.

Colour image

- **CMYK -** a four-color model used in printing by overlaying green-blue (C), purple (M) and yellow (Y) with varying transparency. A layer of black (K) may be added. The CMYK model uses a subtractive (subtractive) color model.

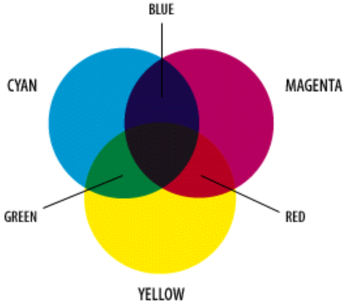
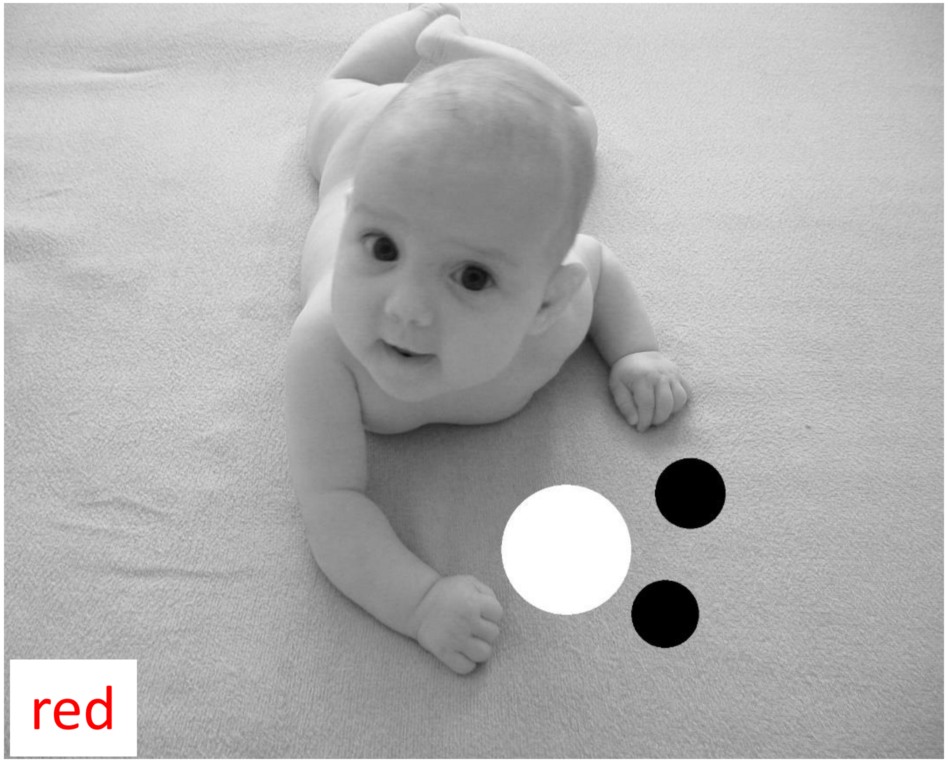
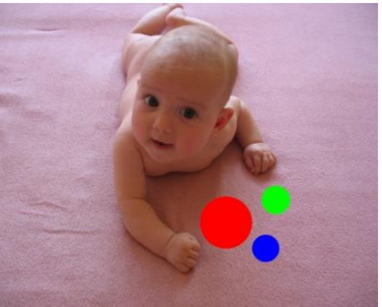


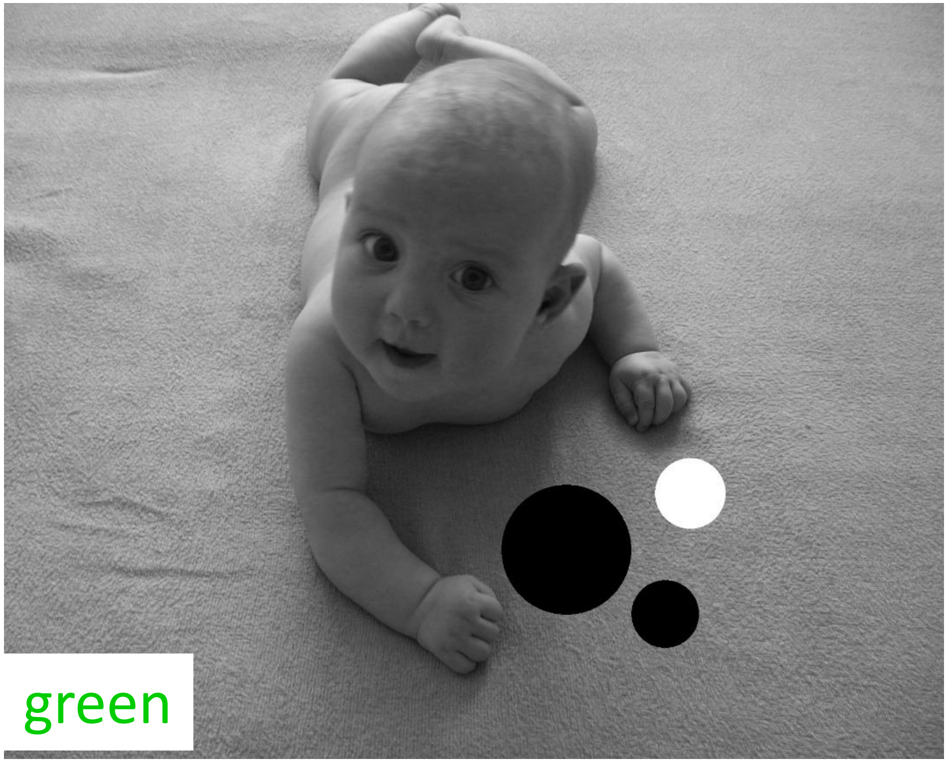
Fig. 5: Colours created by the subtractive model (CMYK) do not look exactly the same as colours from the additive model (RGB). Most importantly, CMYK cannot reproduce the intensity of RGB colours. In addition, the CMYK scale is much smaller than the RGB scale.

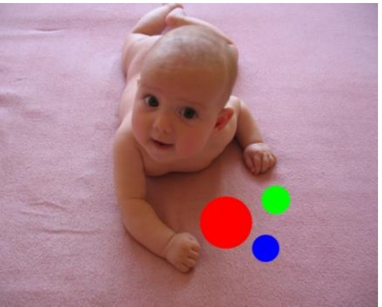
Grey image - explanation



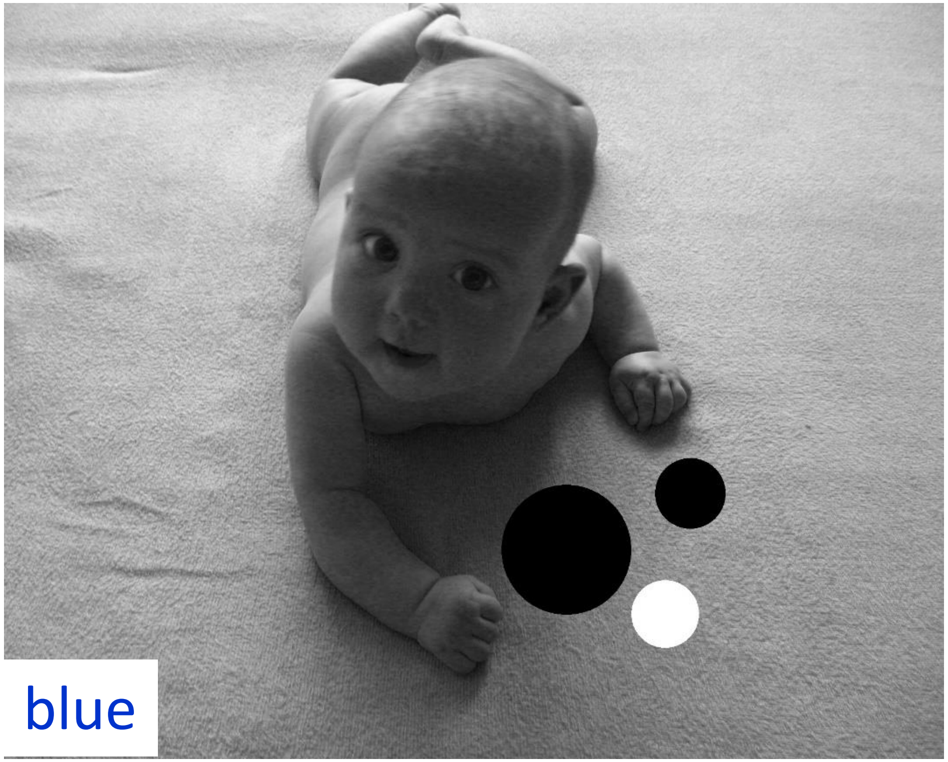


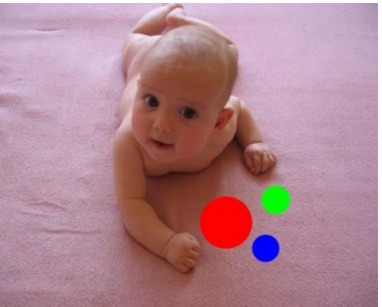
Grey image - explanation



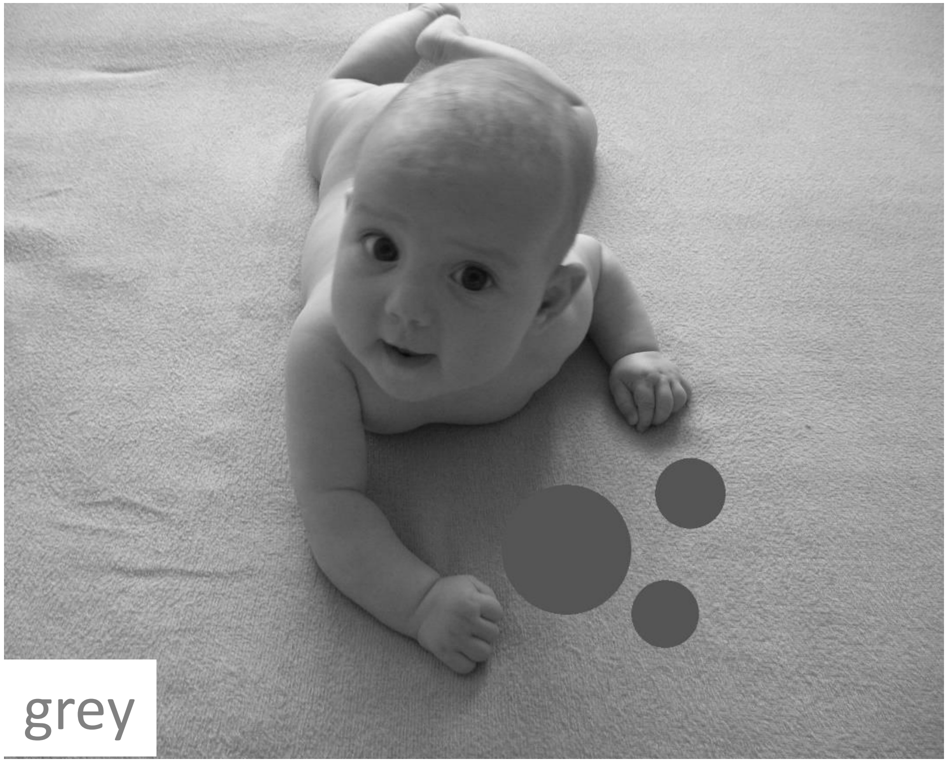


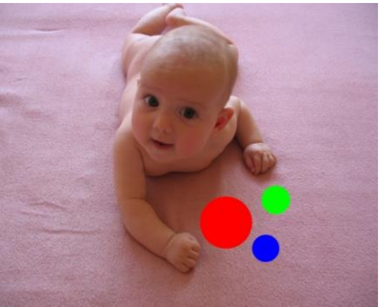
Grey image - explanation





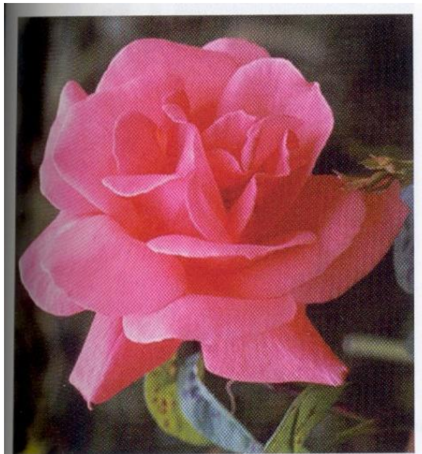
Grey image - explanation



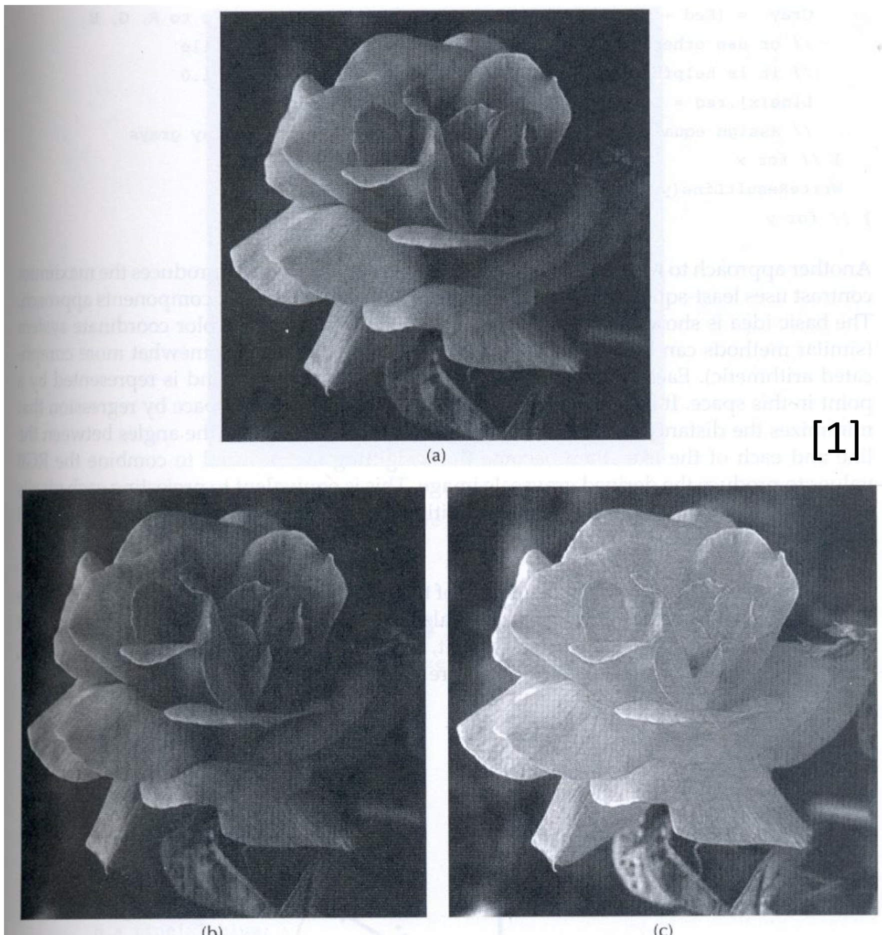


Colour image and its transformation into grey [1]

- Fig. 1.19 - examples of different transformation methods a) - c)
- „rose.tif“ – original image



(a)

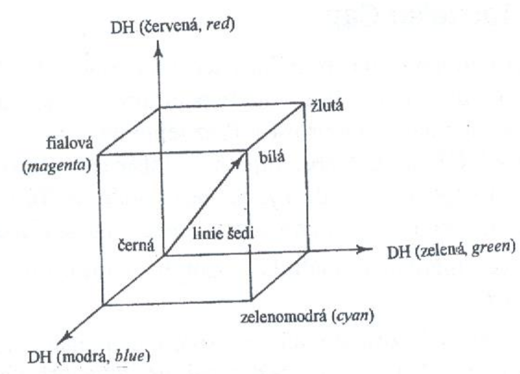


[1]

FIGURE 1.19
Reducing a color image to grayscale (the original image [rose.tif] is shown in Figure 1.11a): (a) average of RGB, (b) luminance (0.25 * red + 0.65 * green + 0.10 * blue), (c) arbitrary weights (0.75 * red + 1.20 * green – 0.65 * blue).

Colour image and its transformation into HSI [3]

- Digital images are usually displayed as additive colour syntheses composed of three so-called primary colours, denoted as R - red, G - green, B - blue.
- The colour cube (Figure 44) shows the relationships between the RGB components for typical colour devices such as a television screen.
- The color cube is defined by the brightness levels of each of the three primary colors. An 8 bits/pixel display has a range of digital values for each color component of 0 - 255 (28). The total color range is 2563 = 16,777,216 color combinations that can be displayed using three-band color synthesis to create RGB.
- Each pixel in the color synthesis display can be represented in three-dimensional space within the color cube in Figure 44. The line joining the origin and the opposite point on the solid diagonal is known as the gray line because the DHs that lie on this line have the same ratio of all three colors.

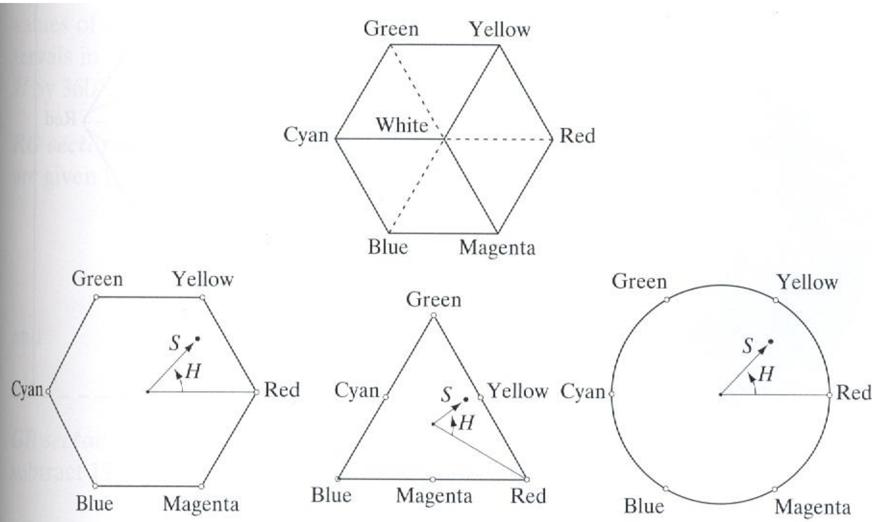


Obr. 44. Krychle barev [3]

Colour image and its transformation into HSI [3]

- Another alternative for colour display is the use of a system called HSI. This abbreviation is based on the English H - hue (hue - expresses the average wavelength of light), S - saturation (saturation - expresses the "purity" of the color relative to the gray level), I - intensity (intensity - expresses the overall brightness of the color).
- The transformation of RGB to HSI can be done e.g. using the hexagonal model, see Figure 45. This transformation is actually a projection of the colour cube onto a plane perpendicular to the solid diagonal of the cube, i.e. the grey line. This plane passes through the outermost point of the cube relative to the origin, see Figure 45. The projection of the cube onto this plane then results in a hexagon as in Figure 45.
- If we move this plane along the grey line towards the origin, we get projections of smaller cubes, hence smaller hexagons. The maximum hexagon has the maximum intensity and the center contains white, the minimum hexagon is one point or origin of the coordinate axes and is black. The other hexagons have centers with values between 0 and various grayscale values.

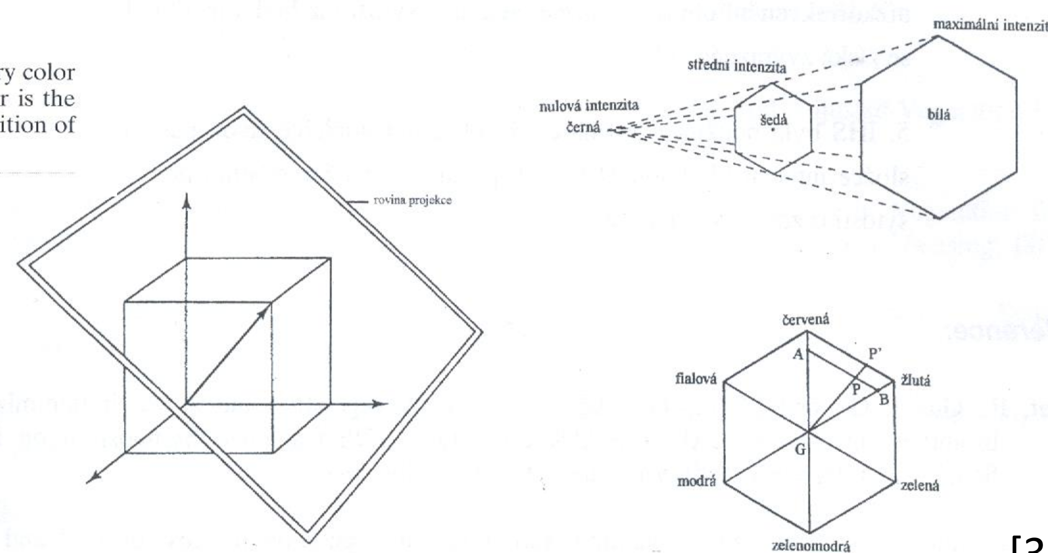
Colour image and its transformation into HSI [3]



a
b c d

FIGURE 6.13 Hue and saturation in the HSI color model. The dot is an arbitrary color point. The angle from the red axis gives the hue, and the length of the vector is the saturation. The intensity of all colors in any of these planes is given by the position of the plane on the vertical intensity axis.

[2]



[3]

Obr. 45. Rovinná projekce krychle do roviny kolmé na tělesovou úhlopříčku a šestiúhelníkový model barev

Colour image and its transformation into HSI [3]

- Figure 45 shows how hue, saturation and intensity are defined:
  - Hue - is defined by the angle around the hexagon $H = \frac{AP}{AB}$
  - Saturation - for this, the further away the point is from G, the greater the saturation. $S = \frac{GP}{GP'}$
  - Intensity - the distance on the gray line from black to a given hexagonal projection
- The HSI transform is often used as an intermediate step for image enhancement, e.g. by the following three steps:
  - first we perform the RGB to HSI transformation, which is done automatically on the computer, then
  - highlight the resulting HSI image, and then
  - then we automatically transform it back to RGB
- The highlight operation in HSI has the advantage that each HSI component can be changed independently without affecting the others. The contrast stretch can be applied to the intensity component and the hue along with the saturation will not change.

Colour image and its transformation into HSI [2]

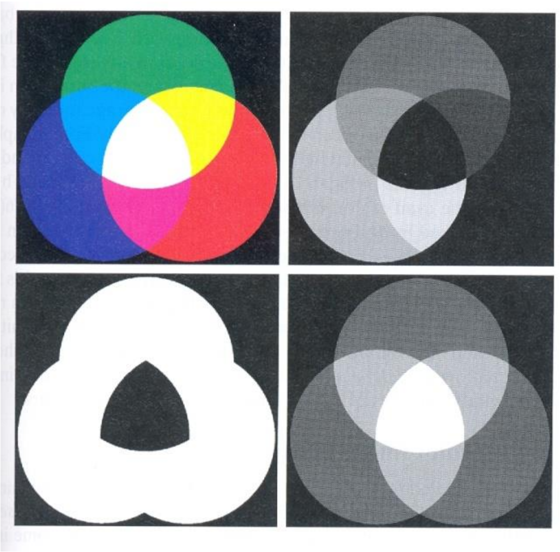
- RGB to HSI transformation, see Fig. 6.16, where R corresponds to 0° for H, i.e. black

$$H = \theta \quad jestliže\, B \le G; \quad 360° - \theta \quad jestliže\, B > G$$

$$\theta = \cos^{-1}\left\{ \frac{\frac{1}{2}\left[(R-G)+[R-B]\right]}{\left[(R-G)^2+(R-B)(G-B)\right]^{1/2}} \right\}$$

$$S = 1 - \frac{3}{(R+G+B)}\left[\min(R,G,B)\right]$$

$$I = \frac{1}{3}(R+G+B)$$



a b
c d

FIGURE 6.16 (a) RGB image and the components of its corresponding HSI image: (b) hue, (c) saturation, and (d) intensity.

[2]

- Transformation HSI to RGB

$$RG\, sektor\, (0° \le H < 120°)$$

$$B = I(1-S), \quad R = I\left[1 + \frac{S\cos H}{\cos(60° - H)}\right], \quad G = 3I - (R+B)$$

$$GB\, sektor\, (120° \le H < 240°)$$

$$H = H - 120°, \quad R = I(1-S), \quad G = I\left[1 + \frac{S\cos H}{\cos(60° - H)}\right], \quad B = 3I - (R+G)$$

$$BR\, sektor\, (240° \le °H \le °360°)$$

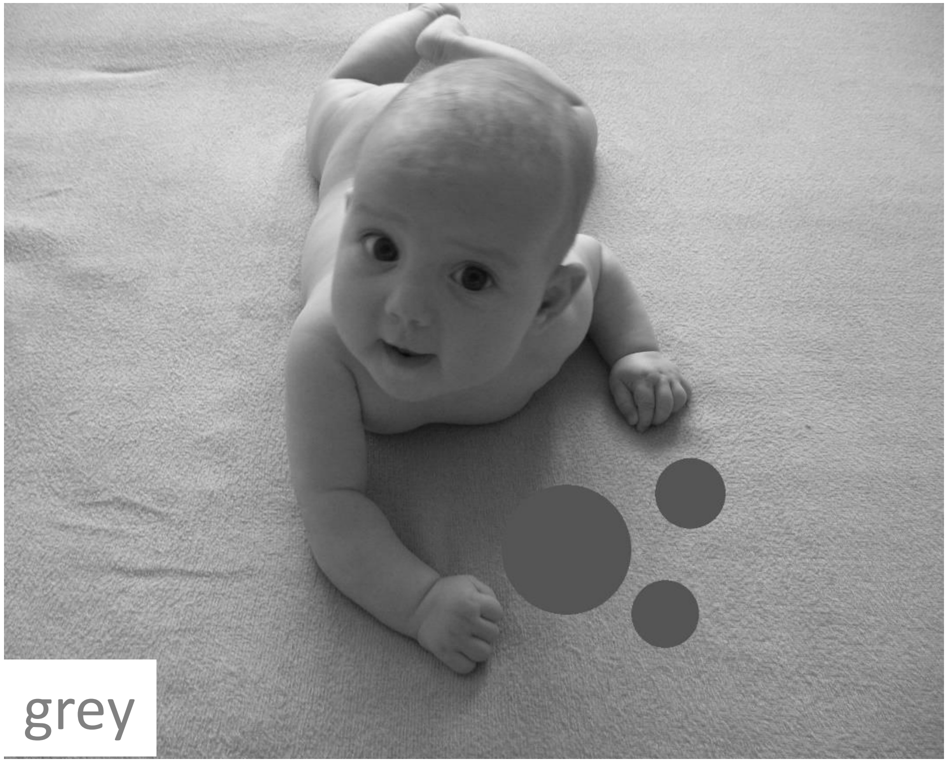
$$H = H - 240°$$

$$G = I(1-S)$$

$$B = I\left[1 + \frac{S\cos H}{\cos(60° - H)}\right]$$

$$R = 3I - (G+B)$$

Binary image - explanation



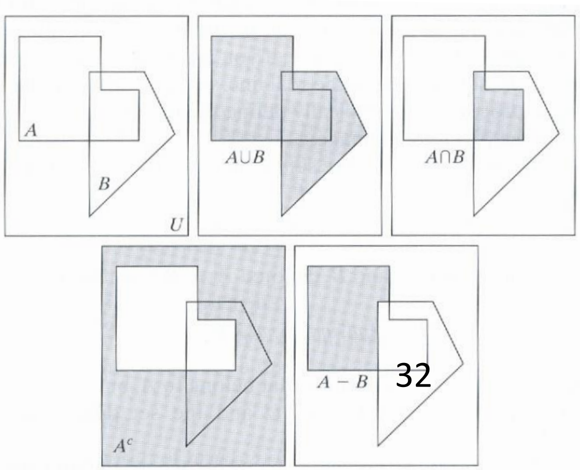
Grey image segmentation on grey image intensity level: <83, 86>

Binary image - explanation

Grey image segmentation on grey image intensity level: <83, 86>

Mathematical and logical operations with images [2]
MATHEMATICAL OPERATIONS

- Two sets A and B are given, whose elements are image points - pixels. Then the following mathematical operations can be defined:
  - Subset - each pixel of set A is also a pixel of set B $A \subseteq B$
  - Unification $C = A \cup B$
  - Intersection $D = A \cap B$
  - Disjunction - sets A and B have empty intersection $A \cap B = \varnothing$
  - Complement - this is a set of pixels that are not part of the given set A A^c
  - Difference of two sets *A* a *B* $A - B = A \cap B^c$
- Fig. 2.31 explains the different mathematical operations. Consider a 2D space defined by the set U in which the various mathematical operations are represented.



a b c
d e

FIGURE 2.31 (a) Two sets of coordinates, *A* and *B*, in 2-D space. (b) The union of *A* and *B*. (c) The intersection of *A* and *B*. (d) The complement of *A*. (e) The difference between *A* and *B*. In (b)–(e) the shaded areas represent the members of the set operation indicated.

[2]

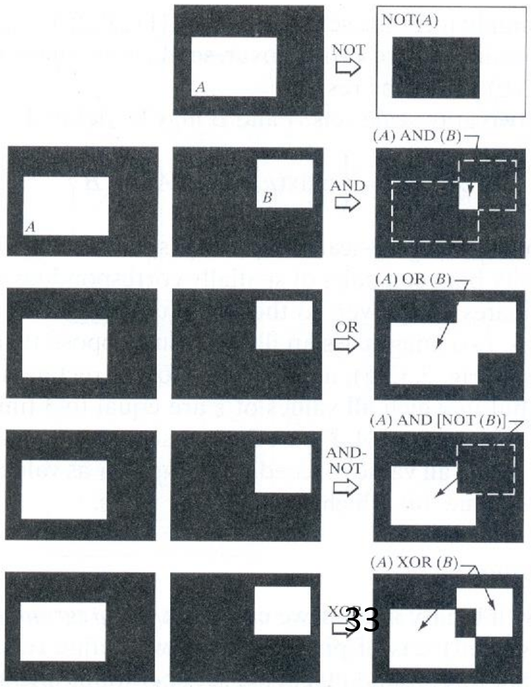
Mathematical and logical operations with images [2]
LOGICAL OPERATIONS

- In the case of binary images, where the pixel value is specified as either 1 (foreground - objects) or 0 (background), the general practice is to replace union, intersection and complement by logical operations - OR, AND, NOT. Where the word "logical" comes from the theory of logic, where 1 - true, 0 - false.
- Consider two regions A and B, which form the foreground with their pixels.
  - The OR of these two files is the set of pixels belonging to either A or B or both.
  - AND results in a set of pixels that are common to both A and B
  - The NOT operation of area A is the set of pixels not belonging to A. In the case of a binary image, these are the pixels that make up the background and foreground given by file B.
  - XOR - exclusive sum - the result is a set of 1-pixels belonging to A or B, but not both.
  - AND-NOT - is defined by the mathematical operation difference, i.e. the result is 1-pixels belonging to A but not to B.
  - It is possible to create any combination of the three basic logical operations OR, AND, NOT.

See Figure 2.33 for an explanation. EXA_02

[2]

FIGURE 2.33 Illustration of logical operations involving foreground (white) pixels. Black represents binary 0s and white binary 1s. The dashed lines are shown for reference only. They are not part of the result.



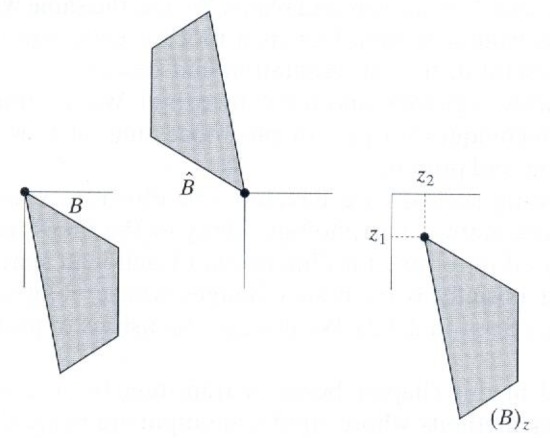
Morphological operations [2] - introduction

- Introduction of the terms 1-pixel set B, mirrored 1-pixel set B^, shifted 1-pixel set (B)z, see Fig. 9.1

a b c

FIGURE 9.1
(a) A set, (b) its reflection, and (c) its translation by z.

[2]



Morphological operations [2] - introduction

- SE
  - structural element, kernel, matrix
  - Certain arrangement of "sub-images" - a cluster of pixels for examination (modification) of the studied image
  - Examples are shown in Figure 9.2. If they are not rectangular, then they are filled in with 0-pixels (background). The centre of the SE is marked with a dot.
  - Its function is explained in Fig. 9.3:
    - object A is also completed to a rectangular shape. The amount of pixels added to the existing object A is controlled by the size of the SE used as follows. The origin of the SE is placed at the boundary of object A and then a number of pixels is added to object A such that the SE does not exceed, i.e. if we had an SE of size 4x4, then one more row of 0-pixels would have to be added to object A.
    - If we have both object A and SE of rectangular shape, then the center of SE is applied successively to each pixel of object A. If SE and A overlap in all pixels, then the center pixel of SE determines the new pixel of the new object. If there is no overlap, then the pixels of object A disappear, effectively eroding object A.

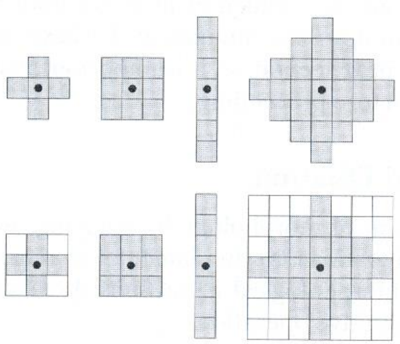
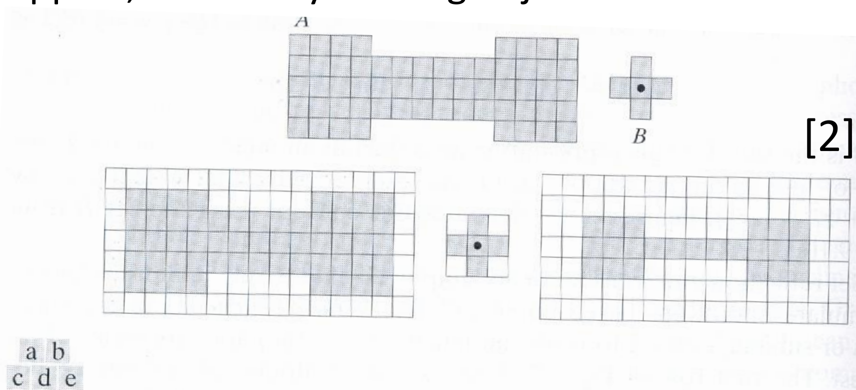


FIGURE 9.2 First row: Examples of structuring elements. Second row: Structuring elements converted to rectangular arrays. The dots denote the centers of the SEs.

[2]



[2]

FIGURE 9.3 (a) A set (each shaded square is a member of the set). (b) A structuring element. (c) The set padded with background elements to form a rectangular array and provide a background border. (d) Structuring element as a rectangular array. (e) Set processed by the structuring element.

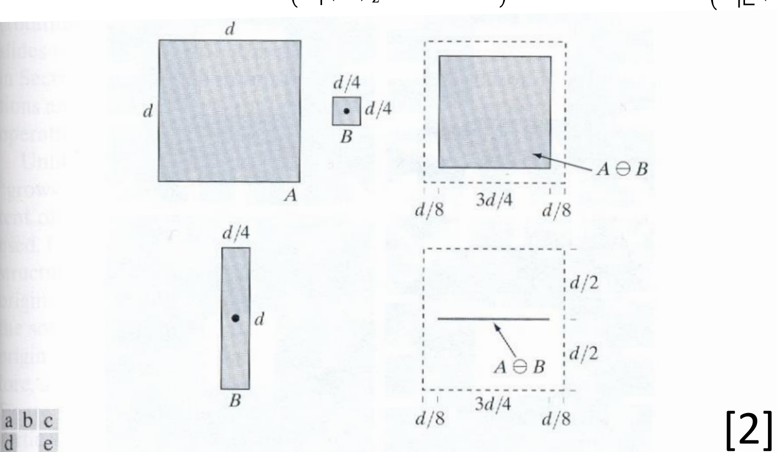
Morphological operations [2]

- Erosion - see Figure 9.4 (explained on the previous slide. The erosion of A by SE B is the set of all points z such that B shifted by z are contained in A)

$$A \ominus B = \left\{ z \middle| (B)_z \subseteq A \right\} anebo\ A \ominus B = \left\{ z \middle| (B)_z \cap A^c = \varnothing \right\}$$

- Dilation - see Figure 9.6 (there is a mirroring of the SE around its origin and a displacement by z. The dilation of A through the SE B is a set of displacements by z such that B^ and A overlap in at least 1 pixel)

$$A \oplus B = \left\{ z \middle| (\hat{B})_z \cap A \neq \varnothing \right\} anebo\ A \oplus B = \left\{ z \middle| \left[(\hat{B})_z \cap A \right] \subseteq A \right\}$$



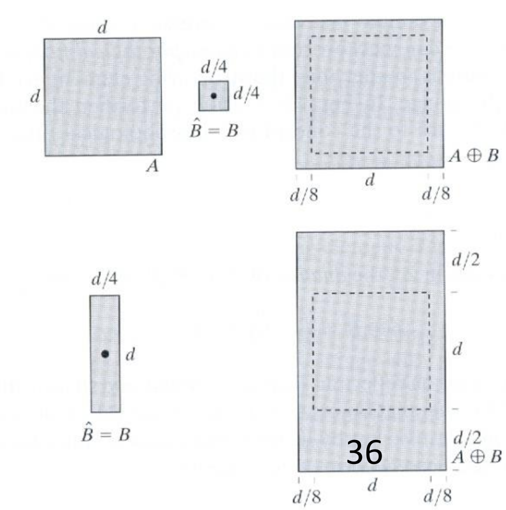
[2]

FIGURE 9.4 (a) Set A. (b) Square structuring element, B. (c) Erosion of A by B, shown shaded. (d) Elongated structuring element. (e) Erosion of A by B using this element. The dotted border in (c) and (e) is the boundary of set A, shown only for reference.

a b c
d e

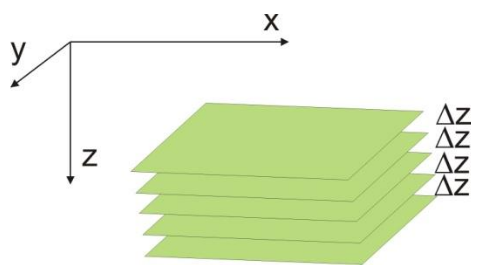
FIGURE 9.6
(a) Set A.
(b) Square structuring element (the dot denotes the origin).
(c) Dilation of A by B, shown shaded.
(d) Elongated structuring element. (e) Dilation of A using this element. The dotted border in (c) and (e) is the boundary of set A, shown only for reference

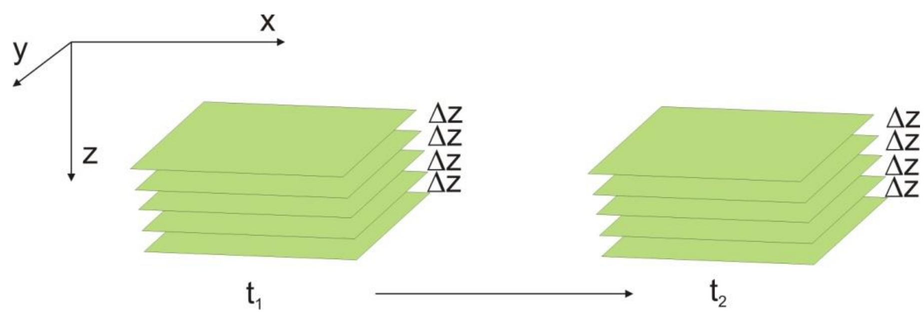
[2]



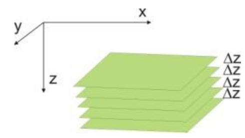
Multi-dimensional "image" - data

- Dimensions:
- xy = 2D
- xyz = 3D
- xyzt = 4D
- xyztλ = 5D

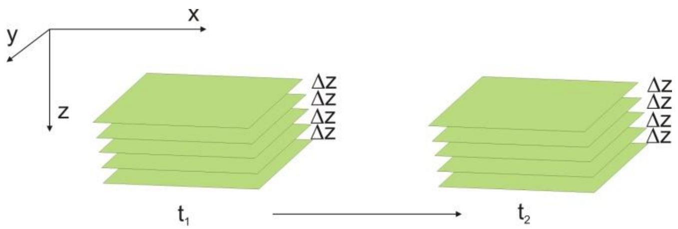




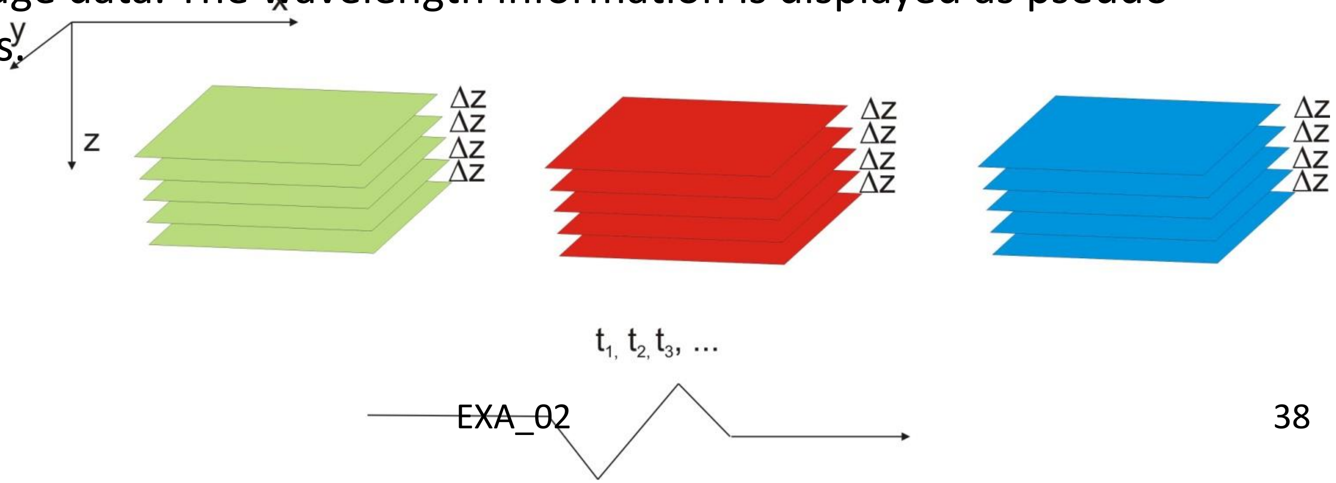
Multi-dimensional "image" - data



- **3D image data**
  - The information from the z-coordinate image can be used to reconstruct a 3-dimensional image.
- **4D image data**
  - 3D image data acquired over time.



- **5D image data**
  - Wavelength is an additional dimension in the case of fluorescence data to 4D image data. The wavelength information is displayed as pseudo-colours.



References used :

[1] John C. Russ, J. Christian Russ: Introduction to image processing and analysis, CRC Press, 2008, USA, ISBN-13: 978-0-8493-7073-1

[2] Gonazalez, R. C., Woods, R. E.: Digital image processing, Pearson Education, Inc., 2008, USA, ISBN: 978-0-13-168728-8

[3] Halounová, L.: Zpracování obrazových dat, ČVUT – Fakulta stavební, Praha, 2009, ISBN 978-80-01-04253-3

[4] manuál NIS-Elements, verze 3.10, 2010, Laboratory Imaging