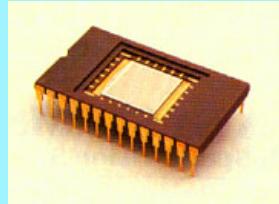
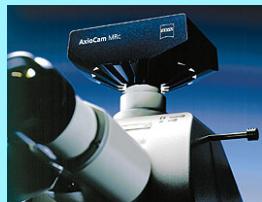


# Image Sensing and Digitization

Jiri Hozman, Ph.D.

Czech Technical University in Prague, Faculty of Biomedical Engineering  
<http://www.fbmi.cvut.cz>



## Image sensing devices I



## Image sensing devices II

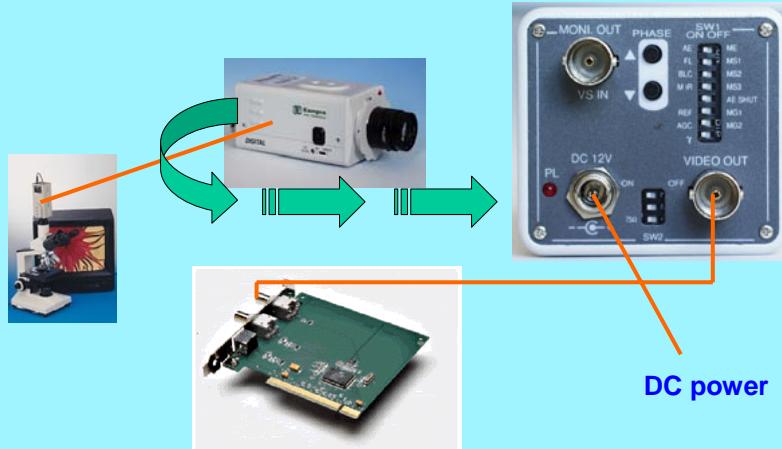


## Image sensing devices III



## Possibilities, how to sense image (1)

- analogue TV videocamera + frame grabber



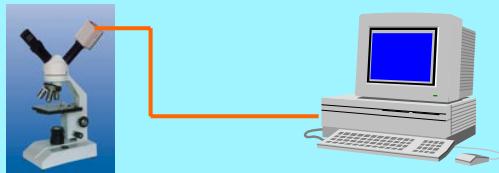
## Possibilities, how to sense image (2)

- digital still camera (DSC)



## Possibilities, how to sense image (3)

- digital videocameras for microscopy with different interface



## Possibilities, how to sense image (4)

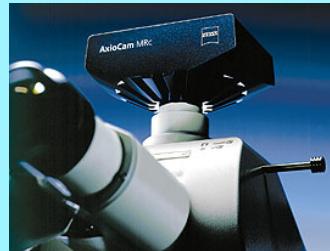
- specialized microscope, USB digital microscope



<http://www.theproscope.com>

## Possibilities, how to sense image (5)

### - specialized complex systems



**High-resolution digital camera systems for microscopy.**

- 1.2 to 5.8 million pixels
- Outstanding image quality
- Color or monochrome
- High sensitivity
- COOLED CCD
- Long integration exposure
- Very easy to learn & operate



## Computers possibilities

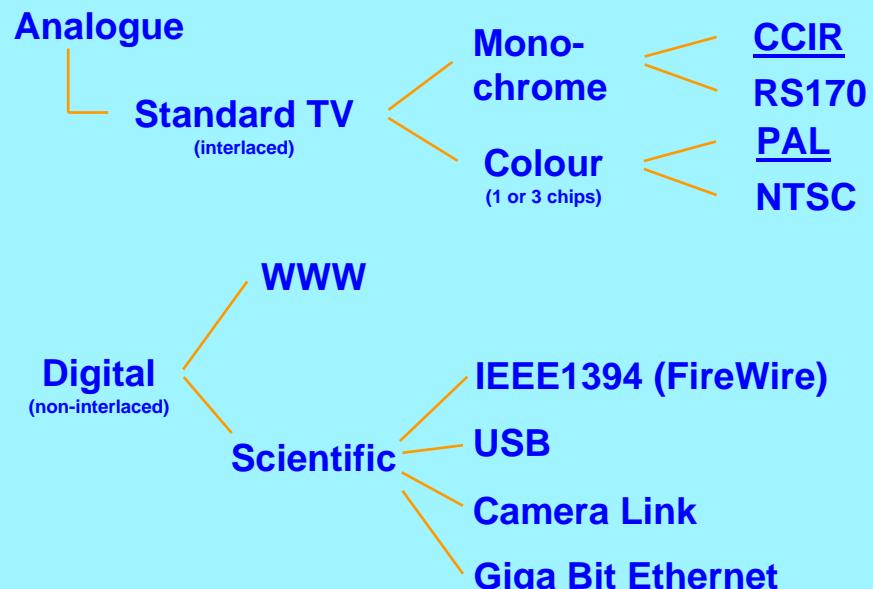
### - standard PC with frame grabber (FG)



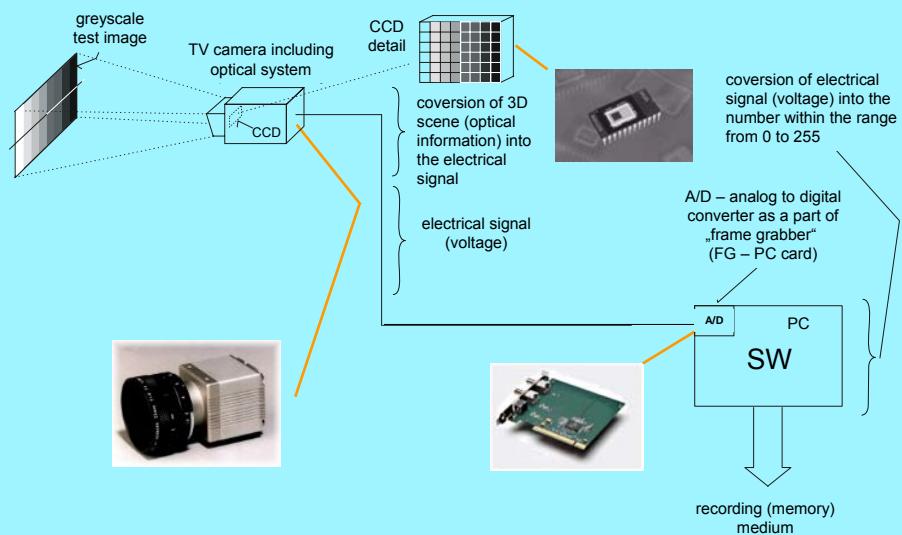
### - laptops with FG (PCMCIA + ext. module)



## Sensing videocameras

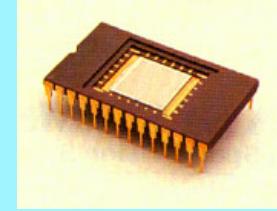
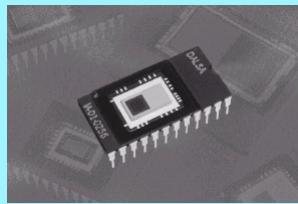


## Basic system configuration

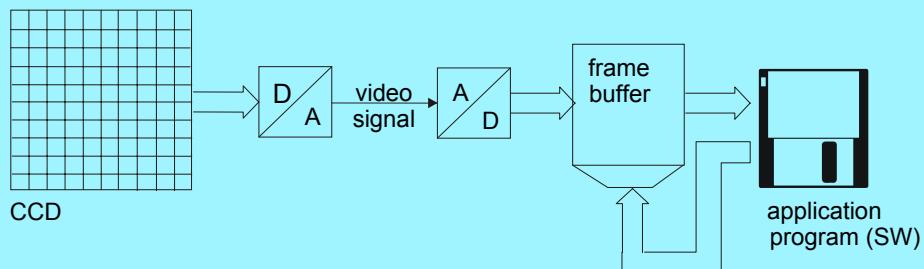


## Image sensing elements

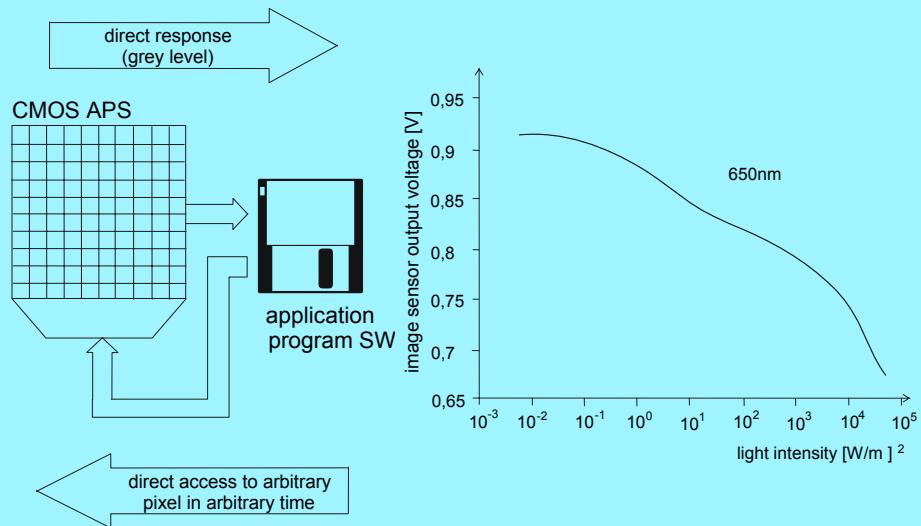
- vacuum tubes
- semiconductor
  - CCD
  - CID
  - CMOS
  - CIS



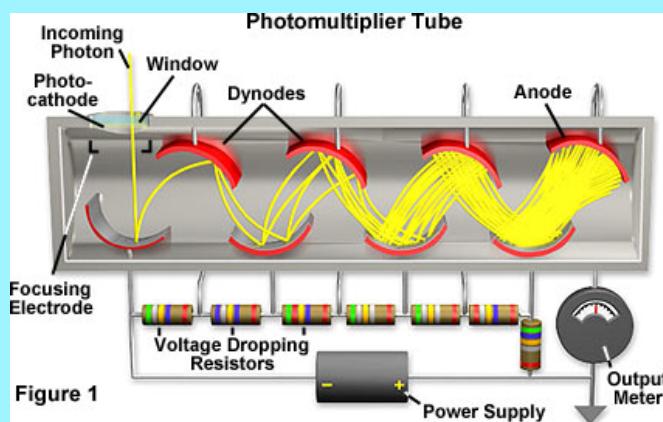
## Basic system configuration with CCD image sensor



## Basic system configuration with CMOS image sensor

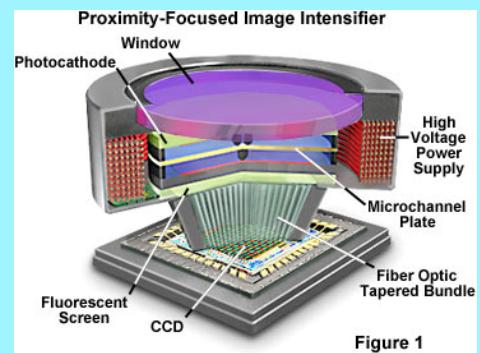
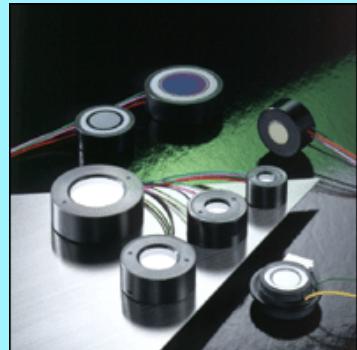


## PMT („photomultiplier tube“ ) (adopted from <http://micro.magnet.fsu.edu>)



PMT are used in confocal microscopes

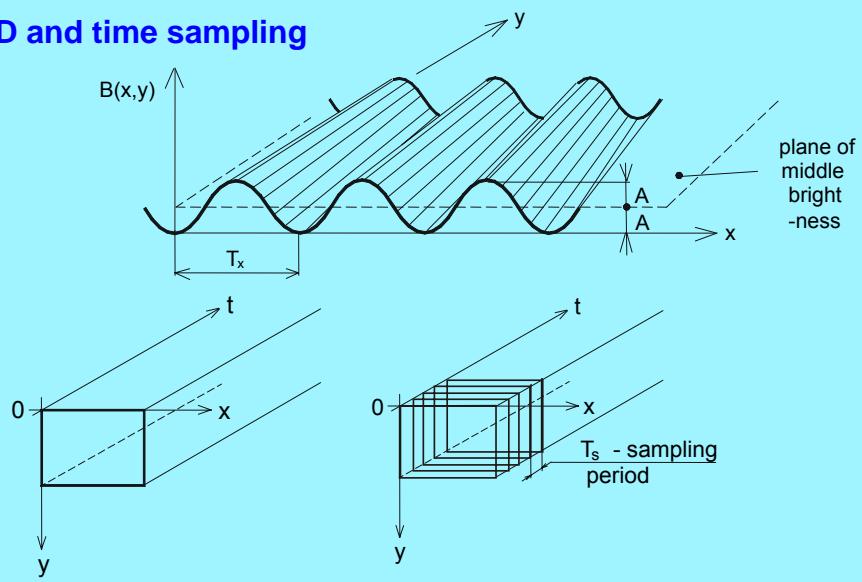
## II („image intensifiers“) - ICCD



adopted from <http://micro.magnet.fsu.edu>

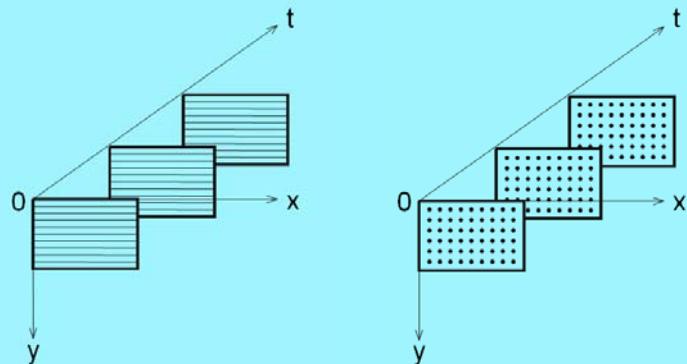
## Image digitization (sampling) I

### - 2D and time sampling



## Image digitization (sampling) II

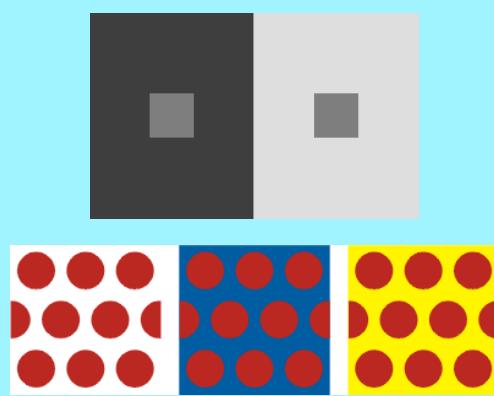
- spatial sampling



## Image digitization (sampling) III

- level digitization (quantization)

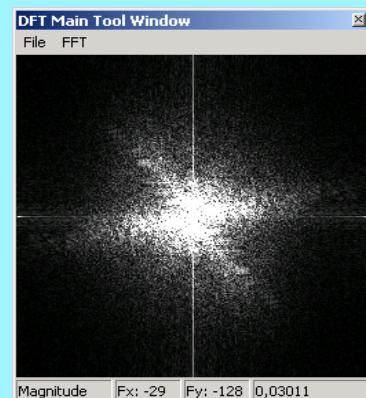
- brightness and contrast perception



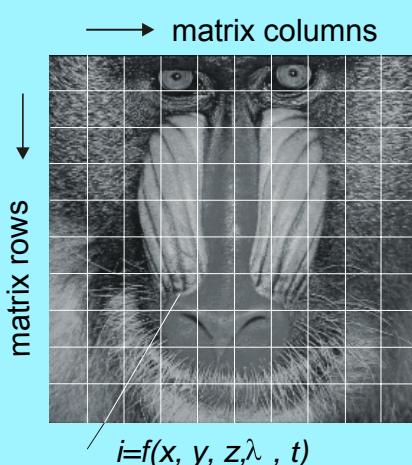
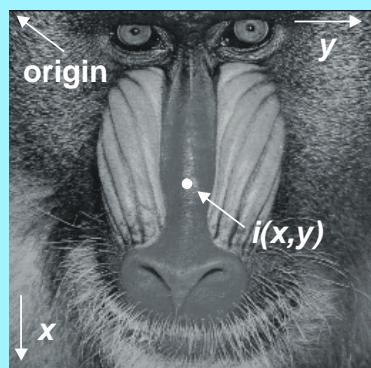
# Fundamentals of Digital Image Processing

Jiri Hozman, Ph.D.

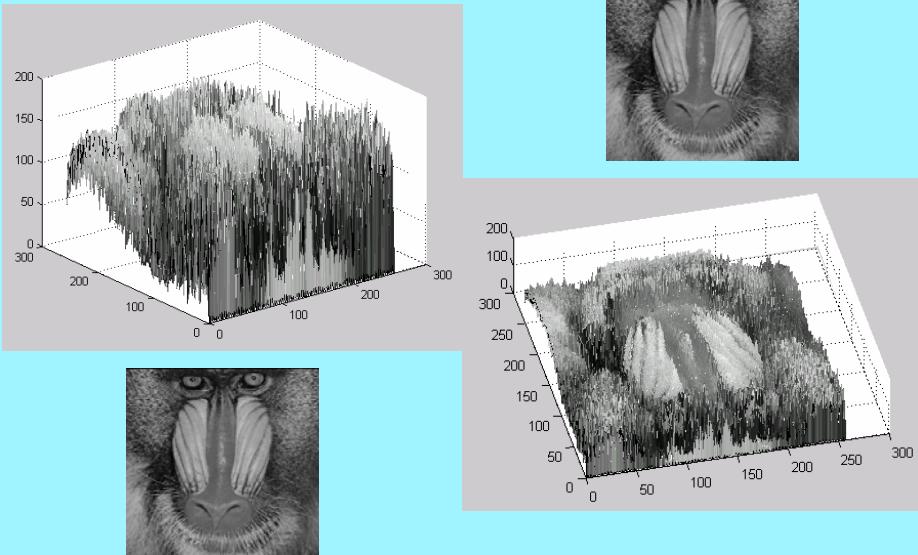
Czech Technical University in Prague, Faculty of Biomedical Engineering  
<http://www.fbmi.cvut.cz>



## Image as two-dimensional matrix



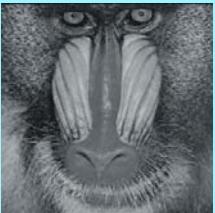
## Image as 3D relief



## Image resolution and number of grey levels

Image resolution	Different number of grey levels	
256 x 256 pixels	2 grey levels	4 grey levels
128 x 128 pixels	8 grey levels	16 grey levels

## Image resolution and number of grey levels

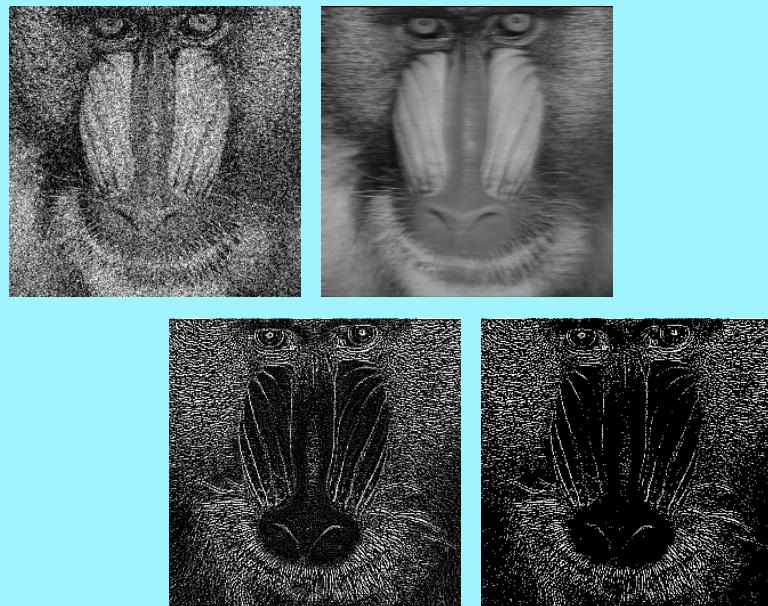
Image resolution	Different number of grey levels	
64 x 64 pixels	32 grey levels	64 grey levels
32 x 32 pixels	128 grey levels	256 grey levels
		
		

## Image processing steps

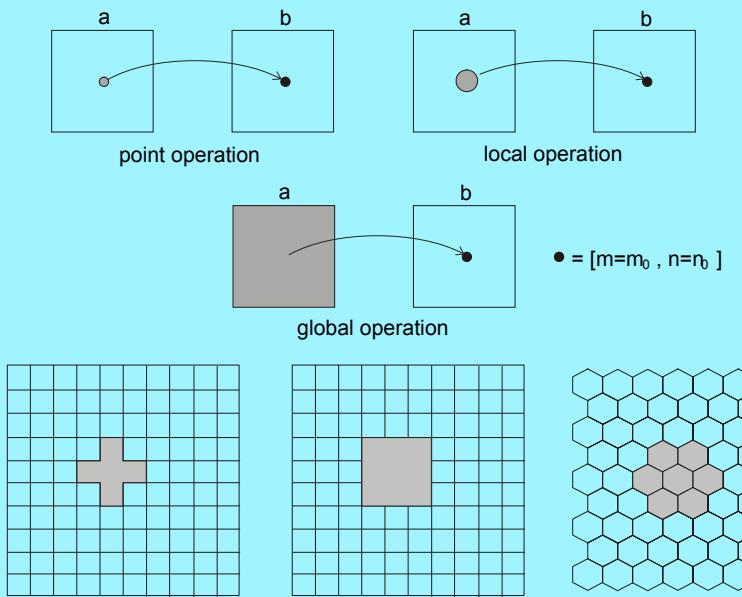
---

- image preprocessing (image enhancement)
- segmentation
- object description (analysis)
- image understanding

## Examples of image operations



## Image operations and pixel neighbourhood



## Image arithmetic operations I

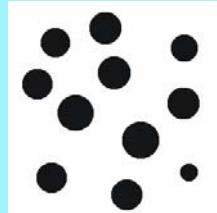
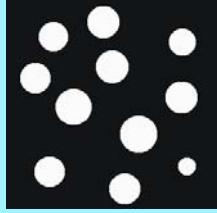
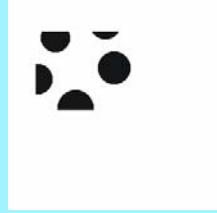
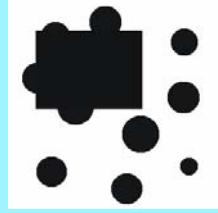
Arithmetic operation between grey level image ("a") and BW ("b") images and greylevel images, (255 (1) corresponds to white, 0 (0) corresponds to black)		
↓ grey level image "a"	↓ binary (BW) image "b"	↓ ADD(a,b) = a + b
↓ SUB(a,b) = a - b	↓ MULT(a,b) = a * b	↓ DIV(a,b) = a / b

## Image arithmetic operations II

Arithmetic operations between grey level ("a") and BW ("b") images and between grey level images, (255 (1) corresponds to white, 0 (0) corresponds to black)		
↓ MIN(a,b)	↓ MAX(a,b)	↓ AVE(a,b) = average
↓ OVERLAY(a,b)	↓ WEIGHT(25% a, 75% b) = 25% a + 75% b	↓ WEIGHT(50% a, 50% b) = 50% a + 50% b

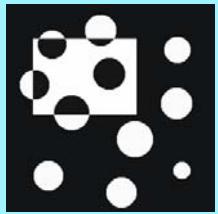
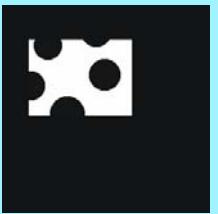
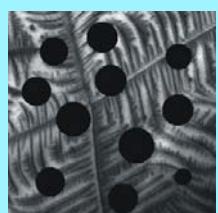
## Image logical (binary) operations I

Logical operations between binary (BW) images (binary point operations) and between grey level ("a") and BW ("b") images (binary value 1 - white, binary value 0 - black)

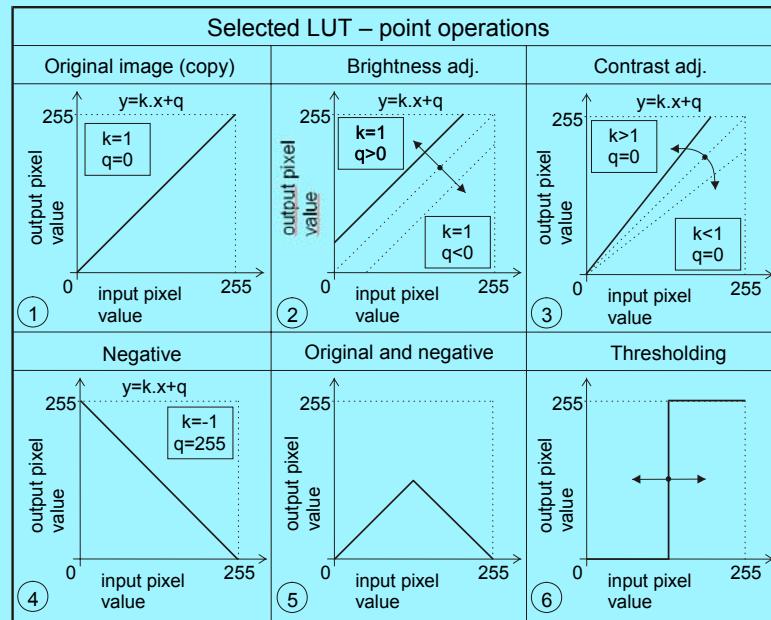
$\Downarrow$ binary (BW) image "a"	$\Downarrow$ binary (BW) image "b"	$\Downarrow$ NOT(a) = $\bar{a}$
		
$\Downarrow$ NOT(b) = $\bar{b}$	$\Downarrow$ OR(a,b) = a + b	$\Downarrow$ AND(a,b) = a * b
		

## Image logical (binary) operations II

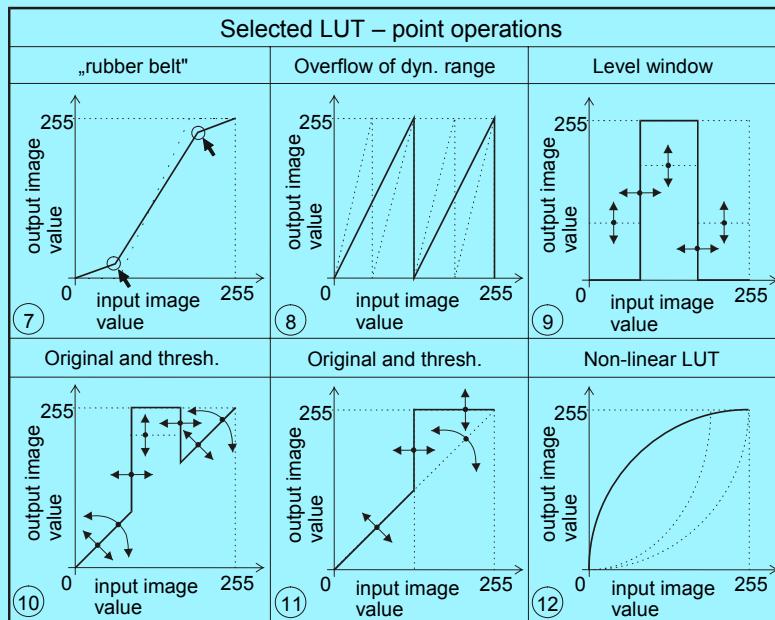
Logical operations between binary (BW) images (binary point operation) and between grey level ("a") and BW ("b") images (binary value 1 - white, binary value 0 - black)

$\Downarrow$ XOR(a,b) = a $\oplus$ b = a $\cdot$ $\bar{b}$ + $\bar{a} \cdot b$	$\Downarrow$ SUB(a,b) = a $\setminus$ b = a - b = a $\cdot$ $\bar{b}$	$\Downarrow$ OR(a,b) = a + b
		
$\Downarrow$ AND(a,b) = a $\cdot$ b	$\Downarrow$ XOR(a,b) = a $\oplus$ b = a $\cdot$ $\bar{b}$ + $\bar{a} \cdot b$	$\Downarrow$ SUB(a,b) = a $\setminus$ b = a - b = a $\cdot$ $\bar{b}$
		

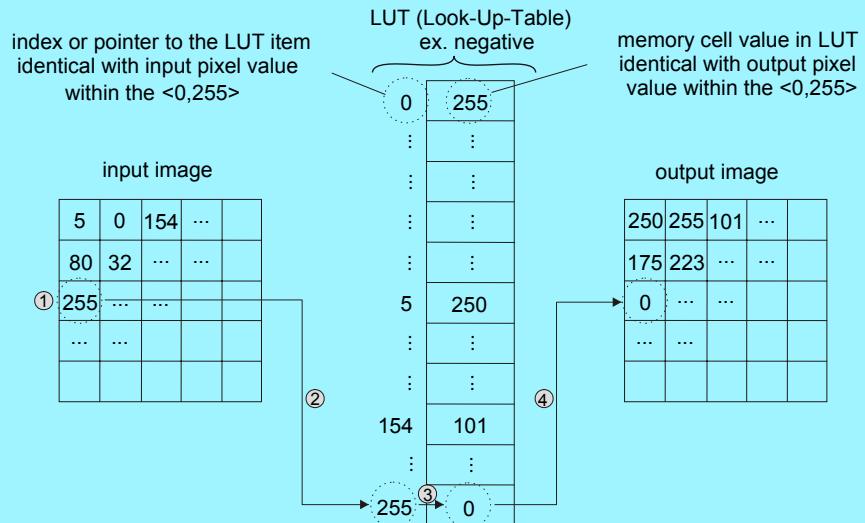
## Look-Up-Tables (LUT) I



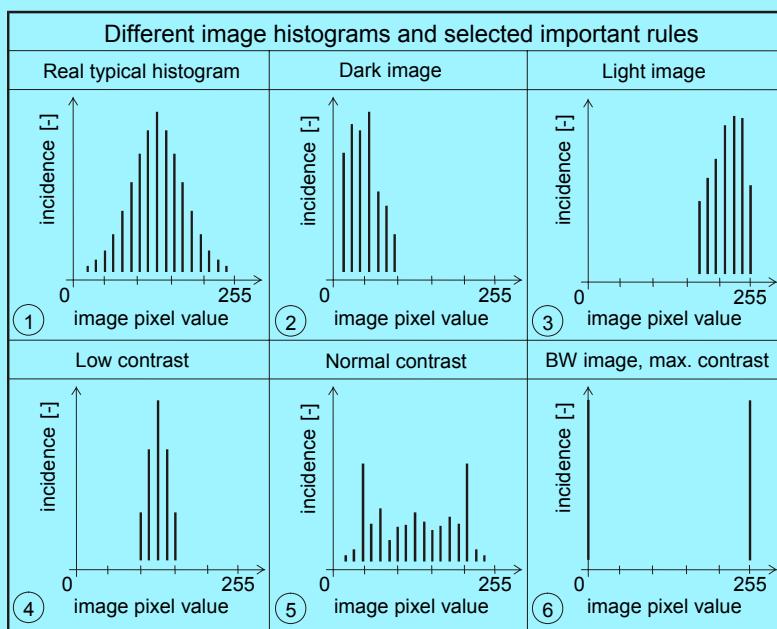
## Look-Up-Tables (LUT) II



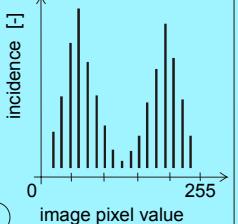
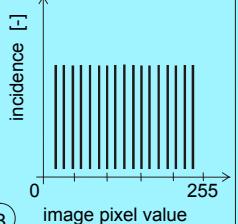
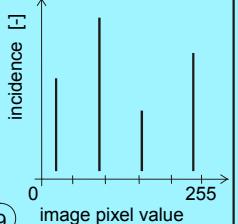
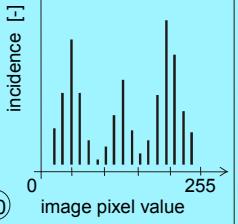
## LUT implementation and application



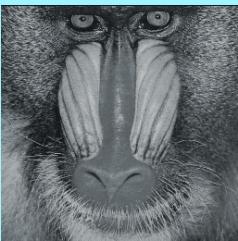
## Image histograms I



## Image histograms II

Different image histograms and selected important rules		
Bimodal histogram	Ideal unreal histogram	4 grey levels in image
 (7)	 (8)	 (9)
Trimodal histogram	Rule No.1 and 2	Rule No. 3 and 4
 (10)	1. Histogram has no relationship to the position of pixel within the image. 2. There is possible to determine an area, that is related to the grey level.  (11)	3. Sum of all incidences is equal to number of pixels within the image. 4. Before the histogram computation there is required to reset 1D array where are saved histogram incidences.  (12)

## Aspects of constant addition to image I

Operat.	Constant addition to orig. image (brightness increasing)				
↓ Subject	Before the operation (input)			After the operation (output)	
Image					
Image data (small detail of the left eye)		56 53 48 49 101 38 22 69 16 36 76 84 196 27 21 22 18 109 14 16 74 27 14 8 22		106 103 98 99 151 88 72 119 66 86 126 134 246 77 71 72 68 159 64 66 124 77 64 58 72	

## Aspects of constant addition to image II

Operat.	Constant addition to the orig. image (brightness incr.)	
↓ Subject	Before the operation (input)	After the operation (output)
LUT	<p>output pixel value</p> <p><math>y = k \cdot x + q</math></p> <p><math>k=1</math> <math>q=0</math></p> <p>input pixel value</p>	<p>output pixel value</p> <p><math>y = k \cdot x + q</math></p> <p><math>k=1</math> <math>q=50</math></p> <p>input pixel value</p>
Histogram	<p>incidence [-]</p> <p>image pixel value</p>	<p>incidence [-]</p> <p>image pixel value</p>

## Aspects of constant subtraction from image I

Operat.	Constant subtraction from image (brightness decreases.)																																																			
↓ Subject	Before the operation (input)	After the operation (output)																																																		
Image																																																				
Image data (small detail of the left eye)	<table border="1"> <tr><td>56</td><td>53</td><td>48</td><td>49</td><td>101</td></tr> <tr><td>38</td><td>22</td><td>69</td><td>16</td><td>36</td></tr> <tr><td>76</td><td>84</td><td>196</td><td>27</td><td>21</td></tr> <tr><td>22</td><td>18</td><td>109</td><td>14</td><td>16</td></tr> <tr><td>74</td><td>27</td><td>14</td><td>8</td><td>22</td></tr> </table>	56	53	48	49	101	38	22	69	16	36	76	84	196	27	21	22	18	109	14	16	74	27	14	8	22	<table border="1"> <tr><td>36</td><td>33</td><td>28</td><td>29</td><td>81</td></tr> <tr><td>18</td><td>2</td><td>49</td><td>0</td><td>16</td></tr> <tr><td>56</td><td>64</td><td>176</td><td>7</td><td>1</td></tr> <tr><td>2</td><td>0</td><td>89</td><td>0</td><td>0</td></tr> <tr><td>54</td><td>7</td><td>0</td><td>0</td><td>2</td></tr> </table>	36	33	28	29	81	18	2	49	0	16	56	64	176	7	1	2	0	89	0	0	54	7	0	0	2
56	53	48	49	101																																																
38	22	69	16	36																																																
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74	27	14	8	22																																																
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18	2	49	0	16																																																
56	64	176	7	1																																																
2	0	89	0	0																																																
54	7	0	0	2																																																

## Aspects of constant subtraction from image II

<b>Operat.</b> $\Rightarrow$	Constant subtraction from image (brightness decreases.)	
<b>↓ Subject</b>	Before the operation (input)	After the operation (output)
LUT		
Histogram		

## Aspects of image multiplication by constant I

<b>Operat.</b> $\Rightarrow$	Image multiplication by constant (contrast increasing)																																																			
<b>↓ Subject</b>	Before the operation (input)	After the operation (output)																																																		
Image																																																				
Image data (small detail of the left eye)	<table border="1"> <tr><td>56</td><td>53</td><td>48</td><td>49</td><td>101</td></tr> <tr><td>38</td><td>22</td><td>69</td><td>16</td><td>36</td></tr> <tr><td>76</td><td>84</td><td>196</td><td>27</td><td>21</td></tr> <tr><td>22</td><td>18</td><td>109</td><td>14</td><td>16</td></tr> <tr><td>74</td><td>27</td><td>14</td><td>8</td><td>22</td></tr> </table>	56	53	48	49	101	38	22	69	16	36	76	84	196	27	21	22	18	109	14	16	74	27	14	8	22	<table border="1"> <tr><td>80</td><td>76</td><td>69</td><td>70</td><td>145</td></tr> <tr><td>54</td><td>31</td><td>99</td><td>23</td><td>51</td></tr> <tr><td>109</td><td>120</td><td>255</td><td>39</td><td>30</td></tr> <tr><td>31</td><td>26</td><td>156</td><td>20</td><td>23</td></tr> <tr><td>106</td><td>39</td><td>20</td><td>11</td><td>31</td></tr> </table>	80	76	69	70	145	54	31	99	23	51	109	120	255	39	30	31	26	156	20	23	106	39	20	11	31
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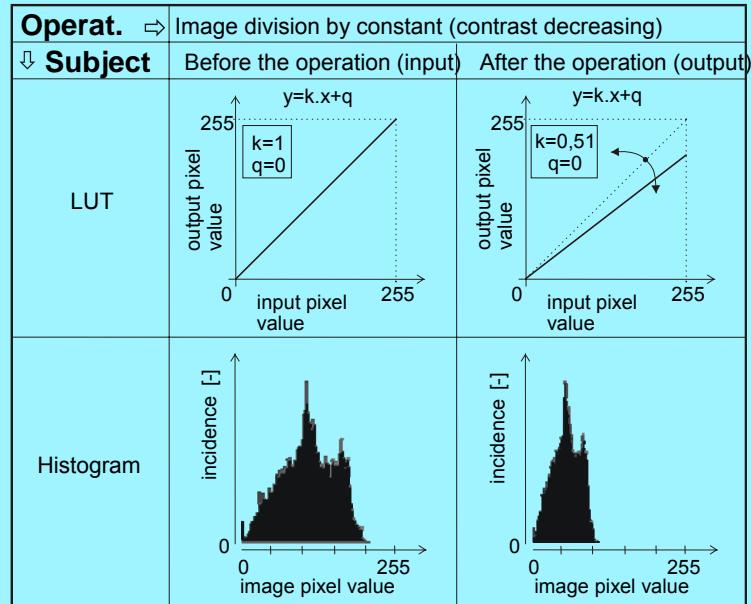
## Aspects of image multiplication by constant II

Operat.	$\Rightarrow$ Image multiplication by constant (contrast increasing)
↓ Subject	Before the operation (input)      After the operation (output)
LUT	
Histogram	

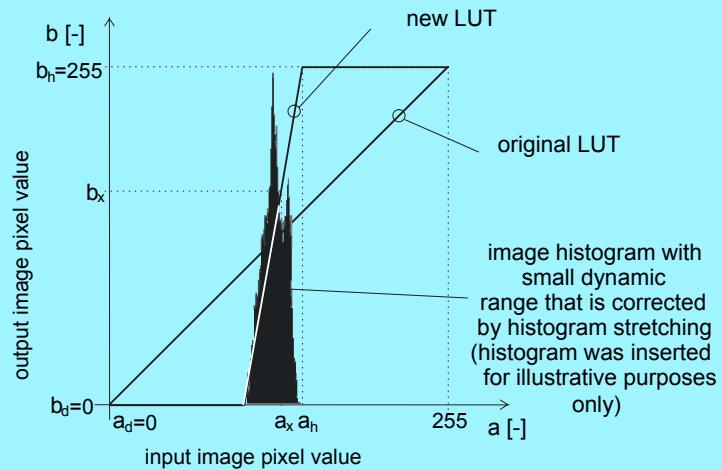
## Aspects of image division by constant I

Operat.	$\Rightarrow$ Image division by constant (contrast decreasing)																																																		
↓ Subject	Before the operation (input)      After the operation (output)																																																		
Image																																																			
Image data (small detail of the left eye)	<table border="1"> <tr><td>56</td><td>53</td><td>48</td><td>49</td><td>101</td></tr> <tr><td>38</td><td>22</td><td>69</td><td>16</td><td>36</td></tr> <tr><td>76</td><td>84</td><td>196</td><td>27</td><td>21</td></tr> <tr><td>22</td><td>18</td><td>109</td><td>14</td><td>16</td></tr> <tr><td>74</td><td>27</td><td>14</td><td>8</td><td>22</td></tr> </table> <table border="1"> <tr><td>29</td><td>27</td><td>25</td><td>25</td><td>52</td></tr> <tr><td>19</td><td>11</td><td>35</td><td>8</td><td>18</td></tr> <tr><td>39</td><td>43</td><td>101</td><td>14</td><td>11</td></tr> <tr><td>11</td><td>9</td><td>56</td><td>7</td><td>8</td></tr> <tr><td>38</td><td>14</td><td>7</td><td>4</td><td>11</td></tr> </table>	56	53	48	49	101	38	22	69	16	36	76	84	196	27	21	22	18	109	14	16	74	27	14	8	22	29	27	25	25	52	19	11	35	8	18	39	43	101	14	11	11	9	56	7	8	38	14	7	4	11
56	53	48	49	101																																															
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29	27	25	25	52																																															
19	11	35	8	18																																															
39	43	101	14	11																																															
11	9	56	7	8																																															
38	14	7	4	11																																															

## Aspects of image division by constant II

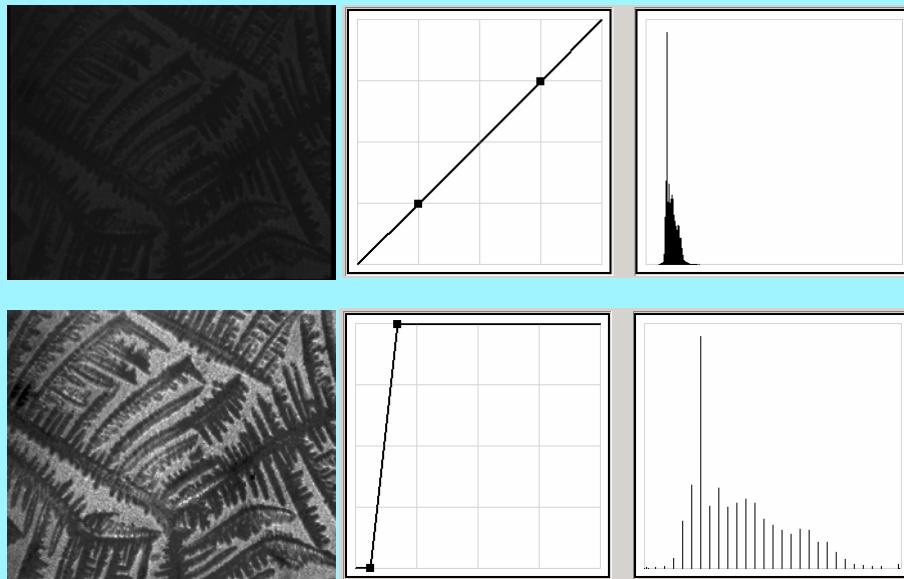


## Histogram stretching I

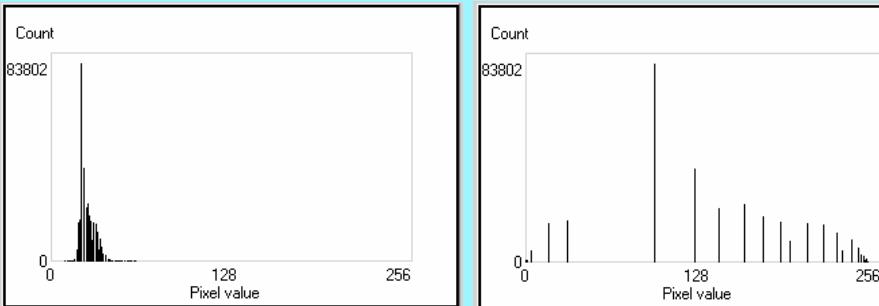


$$b_x = (a_x - a_d) * \left[ \frac{(b_h - b_d)}{(a_h - a_d)} \right] + b_d$$

## Histogram stretching II

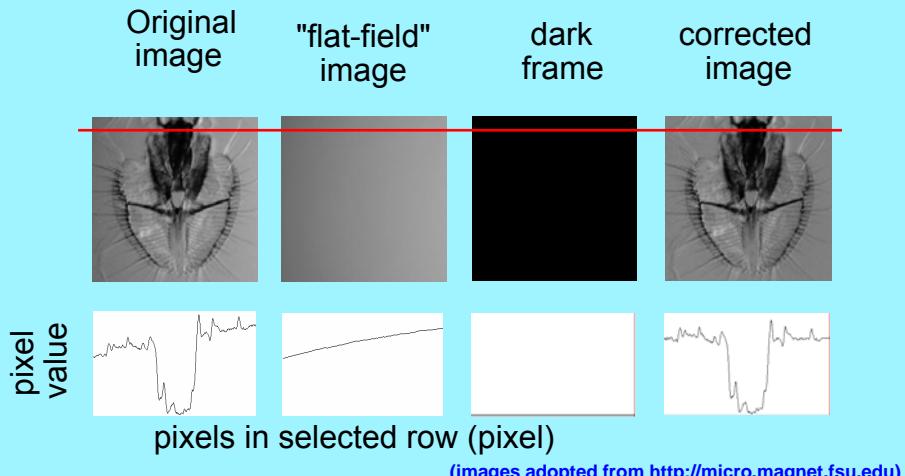


## Histogram equalization

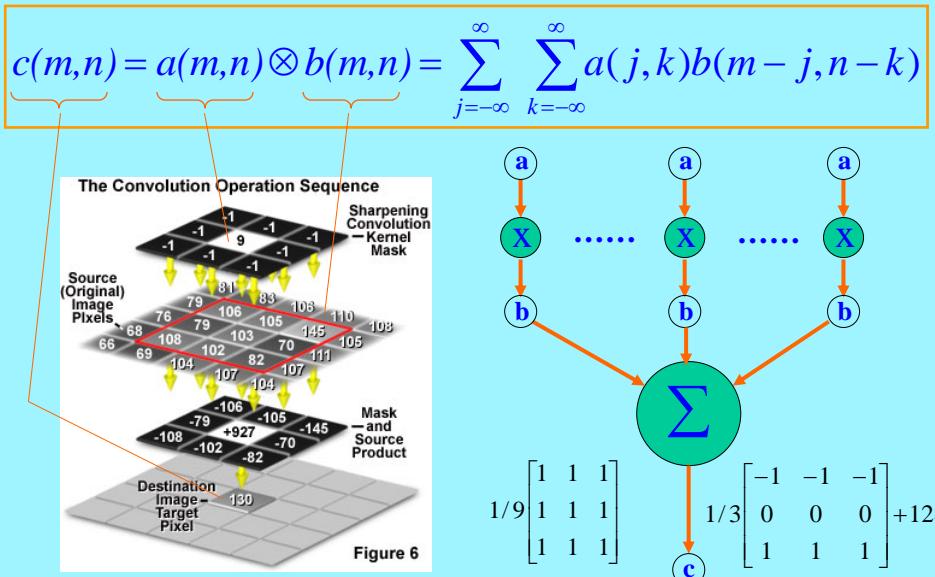


## „Flat-field“ correction

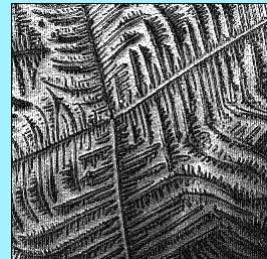
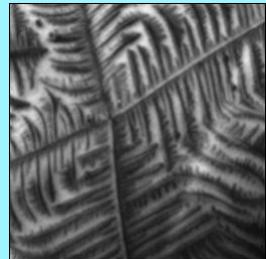
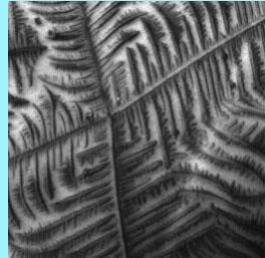
$$I_{corr}(x, y) = \frac{I_{orig}(x, y) - I_{dark}(x, y)}{I_{empty}(x, y) - I_{dark}(x, y)} K$$



## 2D convolution filtration



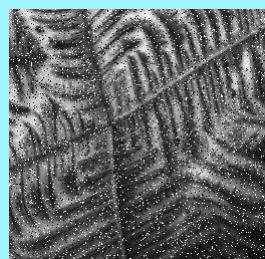
## 2D convolution filtration - examples



$$1/9 \begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{bmatrix}$$

$$\begin{bmatrix} -1 & -1 & -1 \\ -1 & 9 & -1 \\ -1 & -1 & -1 \end{bmatrix}$$

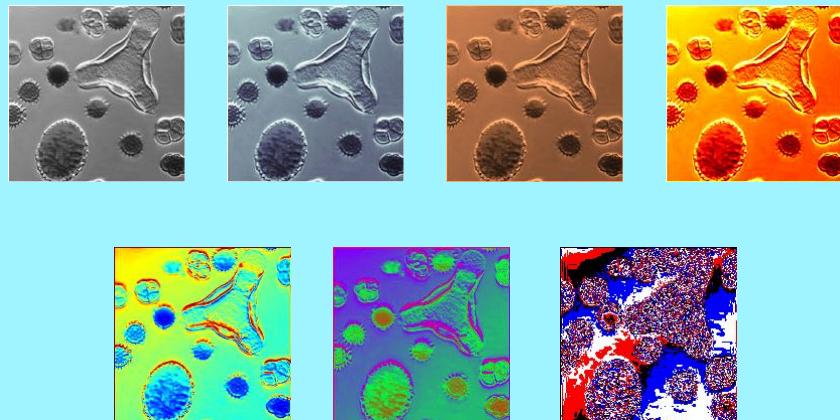
## Median filtration



$$\begin{bmatrix} 10 & 0 & 89 \\ 255 & 56 & 131 \\ 0 & 178 & 255 \end{bmatrix} \rightarrow [0 \ 0 \ 10 \ 56 \ 89 \ 131 \ 178 \ 255 \ 255]$$

↑  
MEDIAN

## Pseudo-colours



## Overview of SW for image processing in Microscopy I

### LUCIA

- <http://www.lim.cz>

### QuickPHOTO MICRO

- <http://www.quickphoto.cz>, <http://www.olympus.cz>

### UCSF, Jain Lab (free)

- <http://www.jainlab.org>

### Morphometrics (free)

- <http://life.bio.sunysb.edu/morph>

## **Overview of SW for image processing in Microscopy II**

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### **The Visualization Toolkit - VTK (free)**

- <http://www.kitware.com>

### **CorelDraw**

- <http://www.corel.com>

### **Photoshop**

- <http://www.adobe.com>

### **Matlab (versus Maple, MathCad)**

- <http://www.mathworks.com>, <http://www.humusoft.cz>

## **SW (free, public domain)**

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### **MIPS (MS Windows) - educational**

- <http://webzam.fbmi.cvut.cz/hozman>

### **Image Magick (MS Windows, Linux, Unix)**

- <http://www.imagemagick.org>

### **XFig (Linux, Unix)**

- <http://www.xfig.org>

## **WWW page with useful links**

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<http://webzam.fbmi.cvut.cz/hozman>

- free download of educational SW MIPS (Microscopy Image Processing Software, with Menu in English and help in Czech)
- free download of lecture (PDF file in Czech),
- free download of presentation (PDF and PPT in English),
- useful links to the image processing topics

**Thank you for your attention**