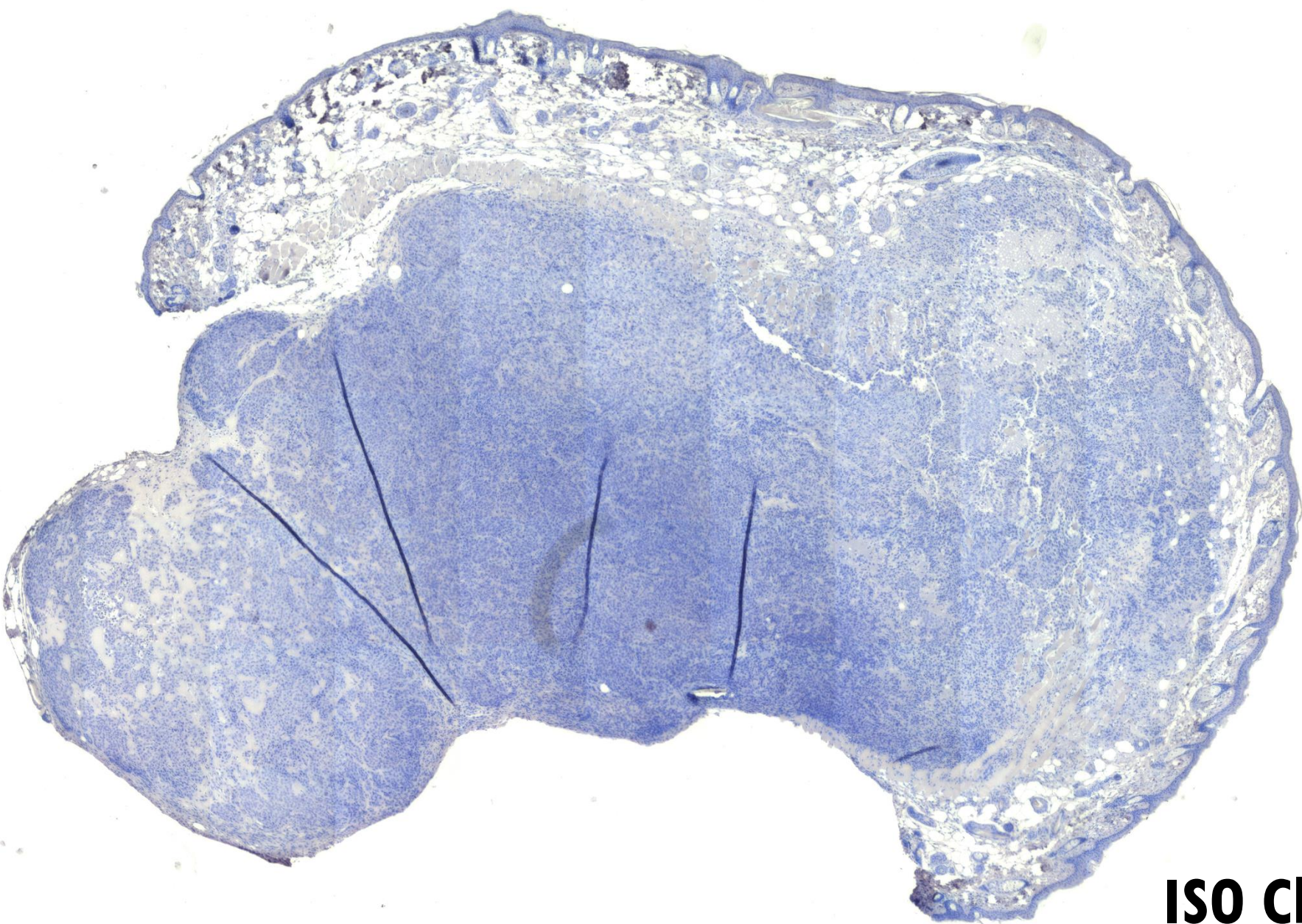


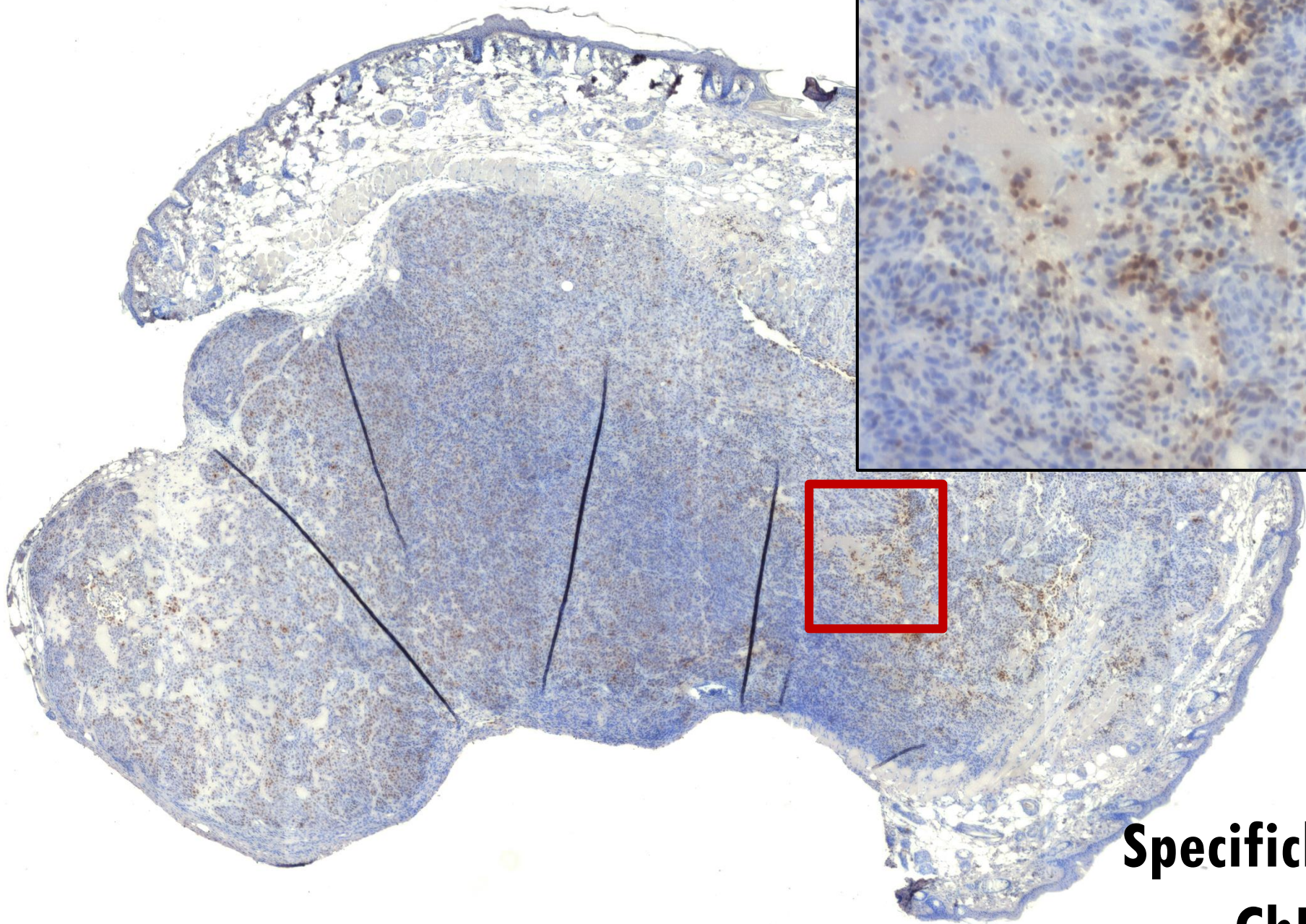
# ZÁKLADNÍ PRINCIPY ANALÝZY OBRAZU

Příklady analýzy  
obrazu

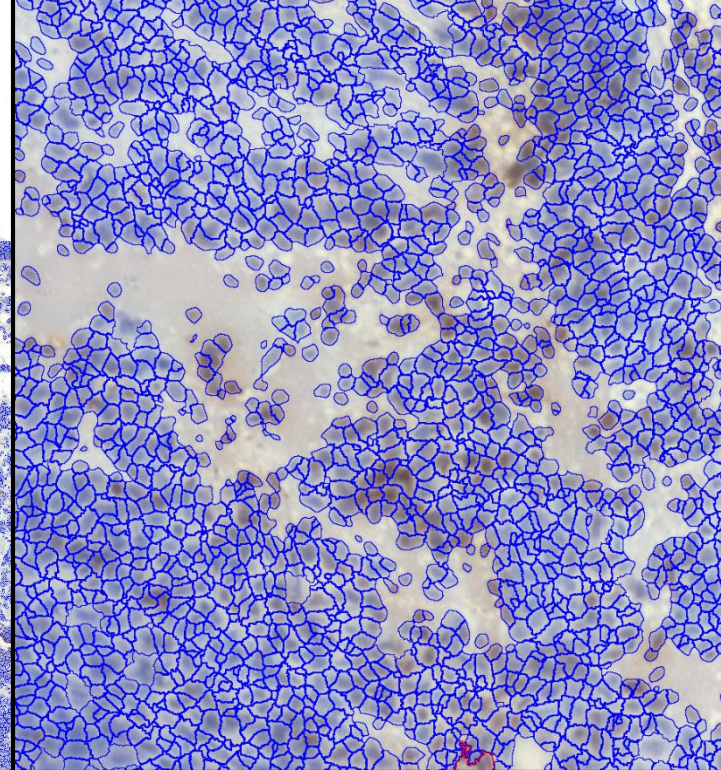
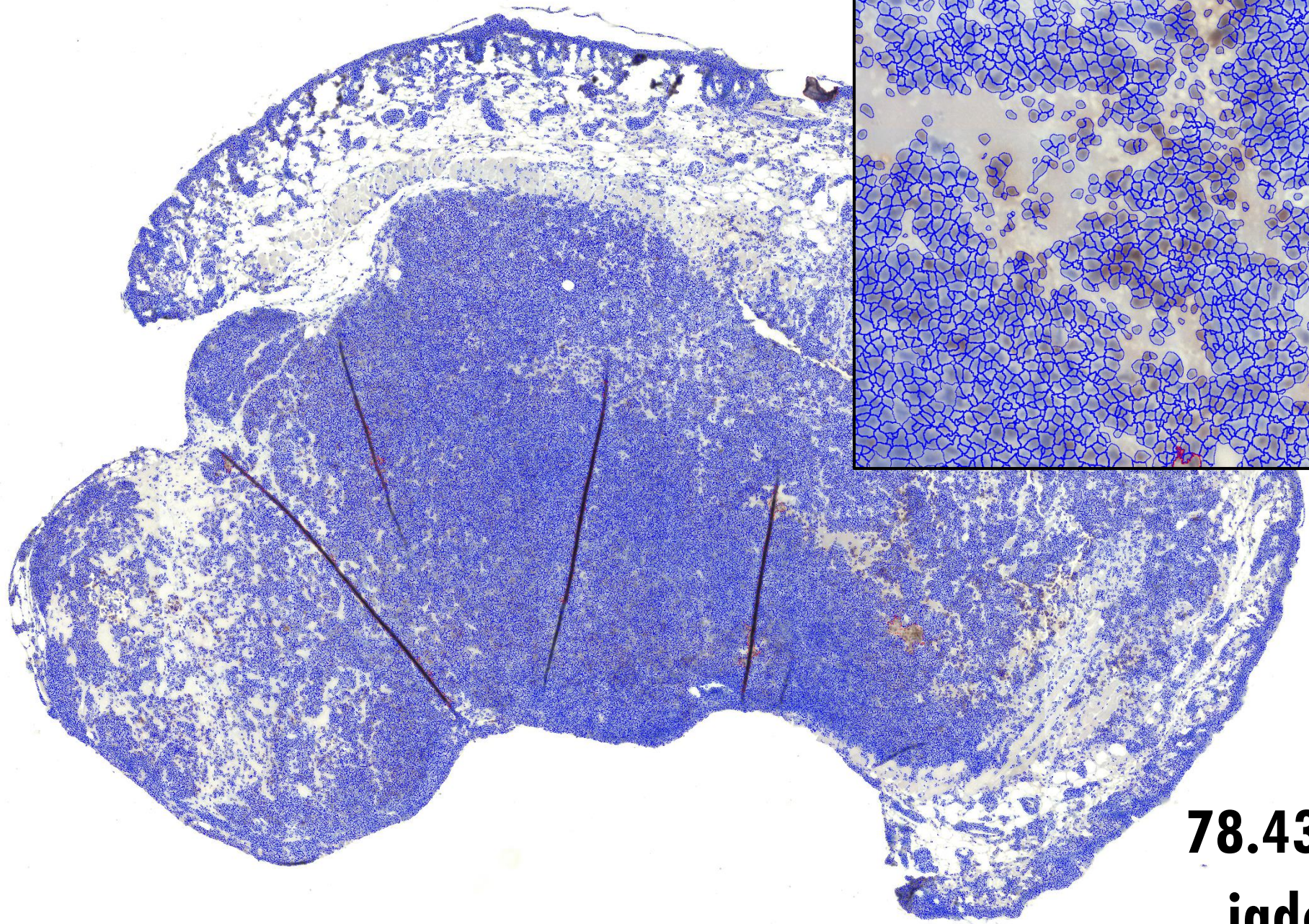
Radek Fedr



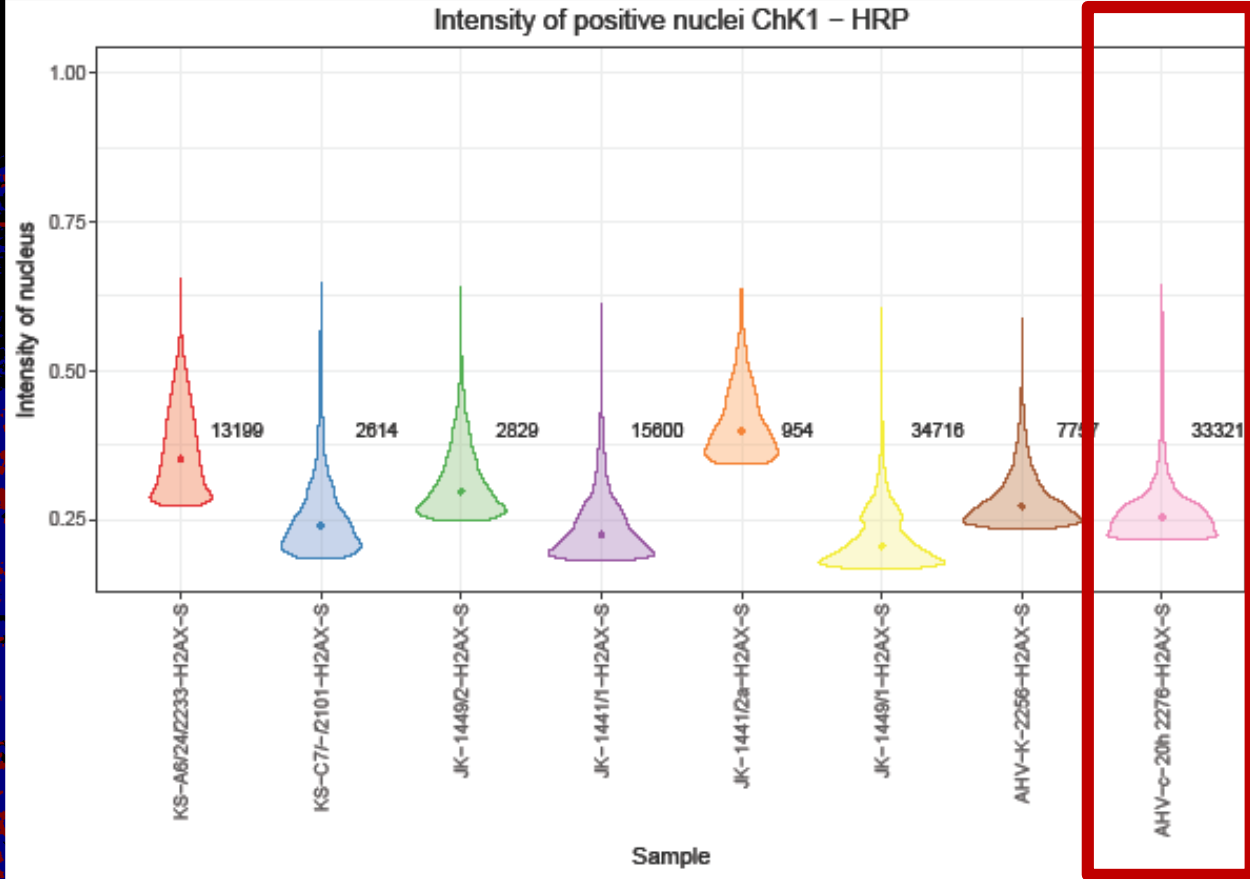
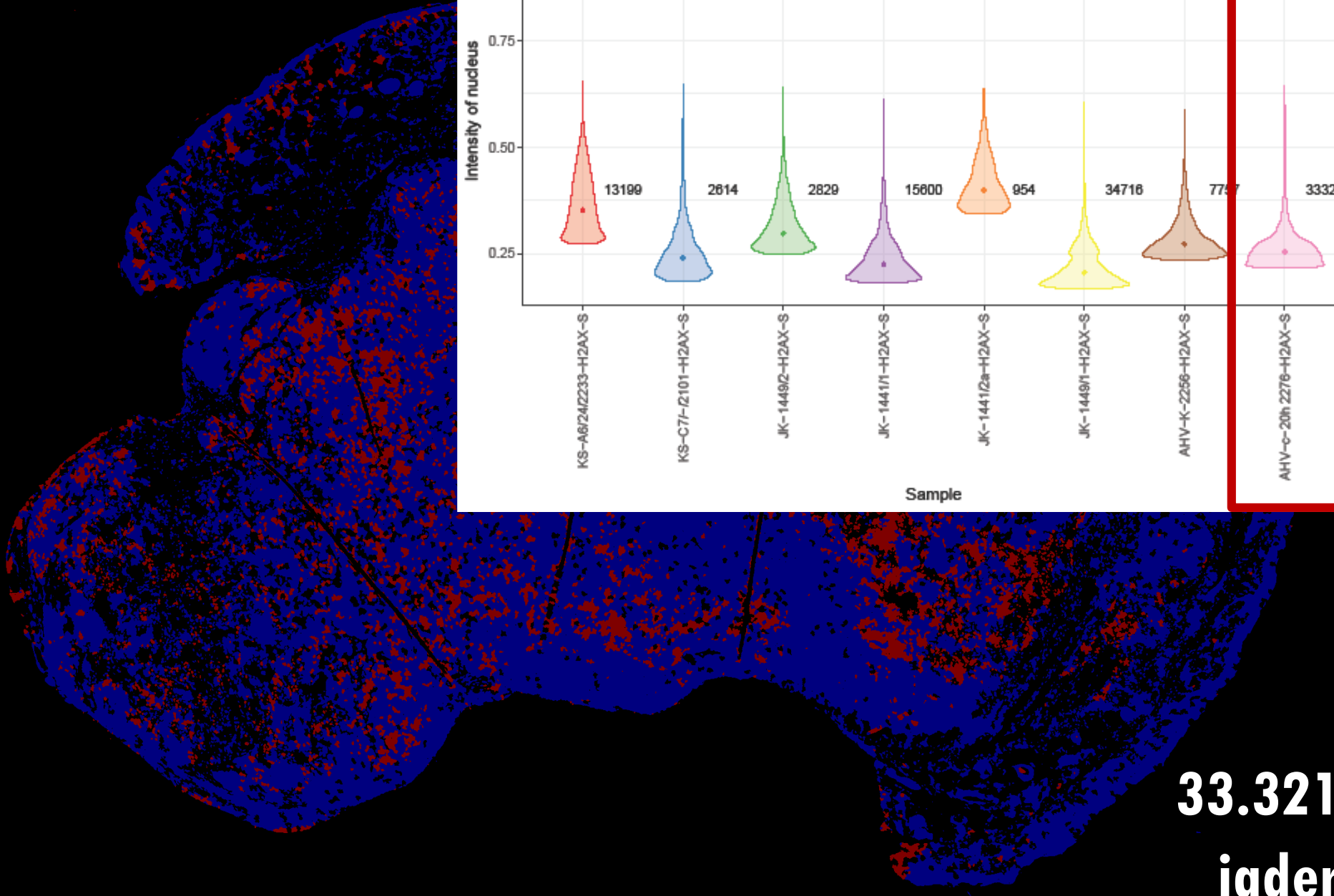
**ISO ChK1**



**Specifický  
ChK1**



**78.436**  
**jader**



**33.321**  
**jader**

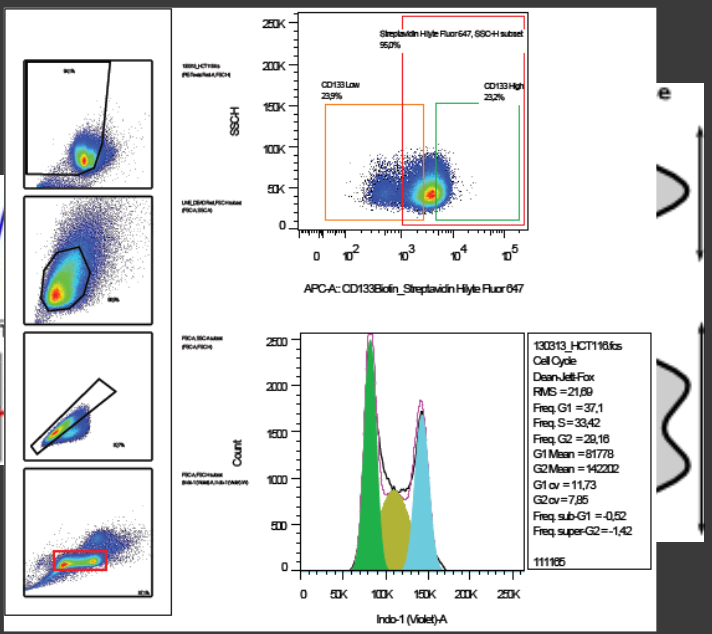
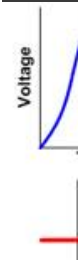
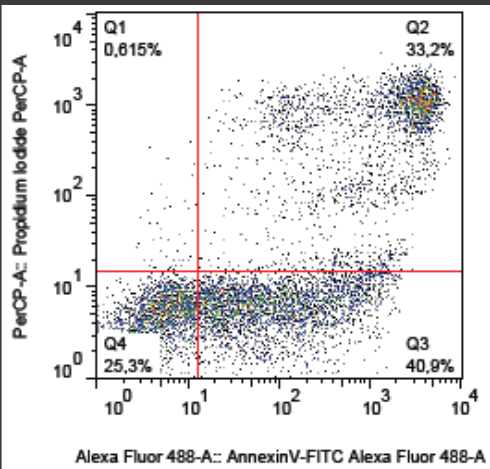
# ANALÝZA TKÁNÍ

- 16 tkáňových řezů (8 ISO + 8 specifické)
- 23,3 GB dat obrazů
- 200 zorných polí/řez
- 5 684 588 jader
- 1,5h analýza obrazů
- 36 parametrů/jádro
- 40 GB dat celkem



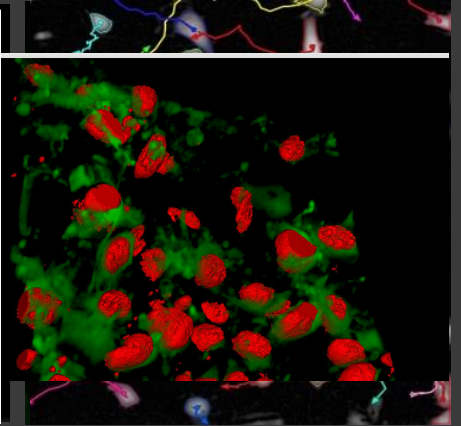
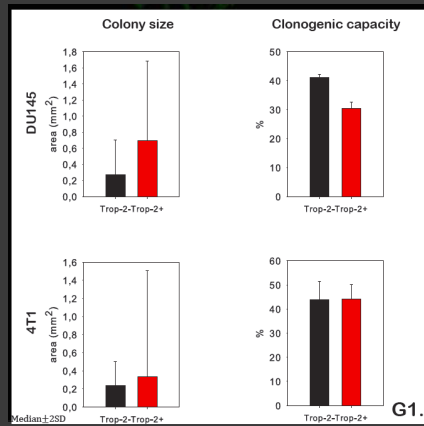
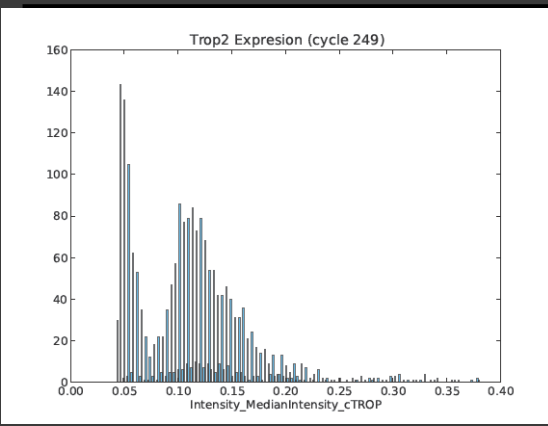
# PRŮTOKOVÁ CYTOMETRIE

- Pulz
- Intenzita - částice
- Distribuce
- Histogram/Bodový graf
- End-point



# OBRAZOVÁ CYTOMETRIE

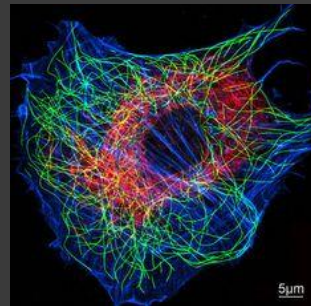
- Pixel
- Intenzita - pixel
- Distribuce
- Obraz, Projekce
- Graf, Model
- Tracking/Sledování



# SROVNÁNÍ

## Mikroskopie

Ukázka



Vzorek

2D, 3D

Rozlišení

100nm

Fluorescenční parametry

1-5

Rychlost

100 buněk/s

Data

100GB

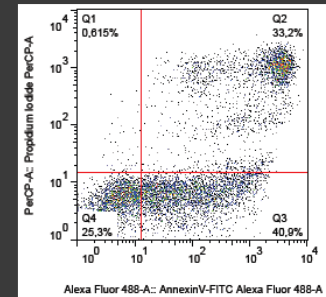
Kvantifikace

Přesná

Počet buněk

$10^1$ - $10^6$

## Průtoková cytometrie



Suspenze

1 částice/1µm

10+

10000 buněk/s

100MB

Přesná

$10^4$ - $10^7$



# POSTUP

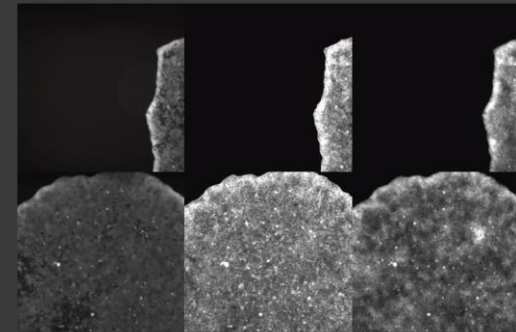
## Příprava vzorku



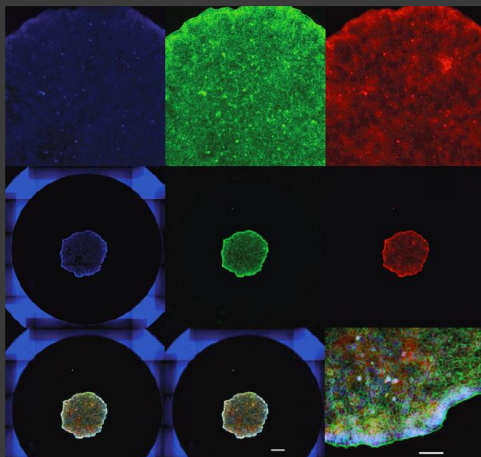
## Snímání



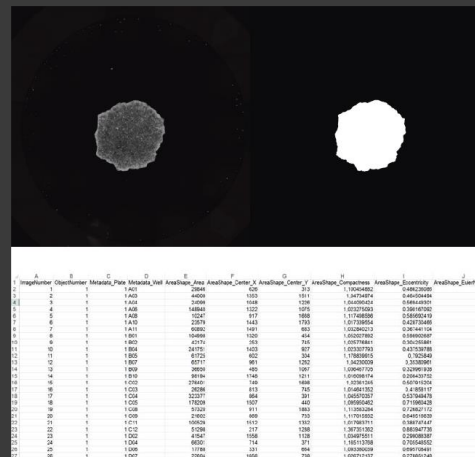
## Uložení dat



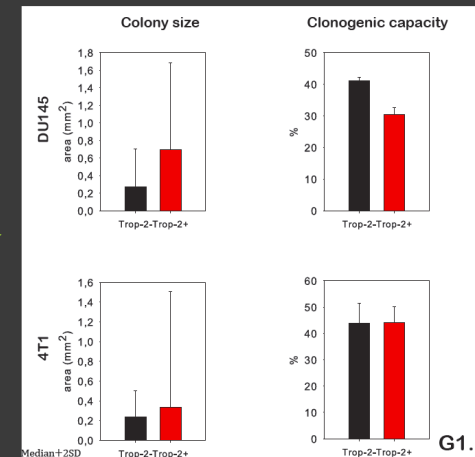
## Zpracování



## Analýza



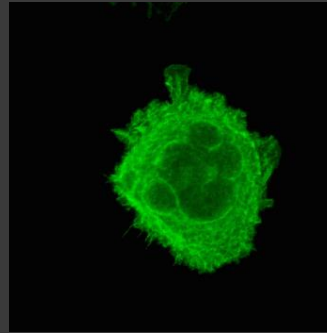
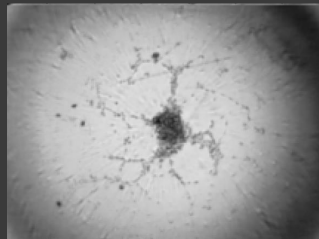
## Vyhodnocení dat



# PŘÍPRAVA A SNÍMÁNÍ

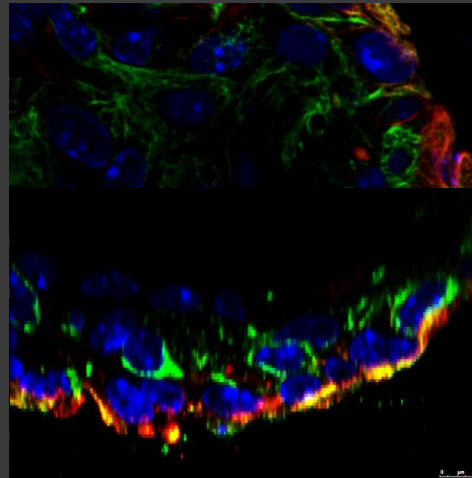
## Experiment

- živé
- fixované
- 2D
- 3D



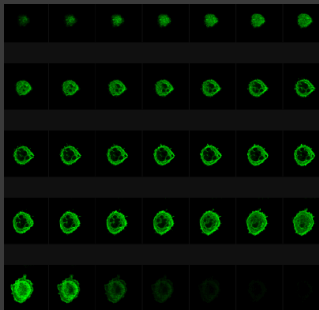
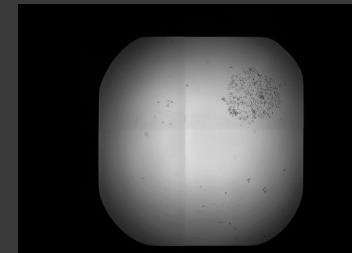
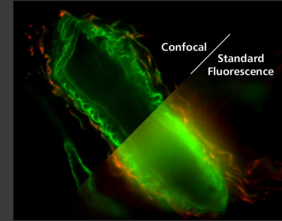
## Vzorek

- suspenze
- monovrstvy
- kokultivace
- tkáň
- sferoidy
- organoidy

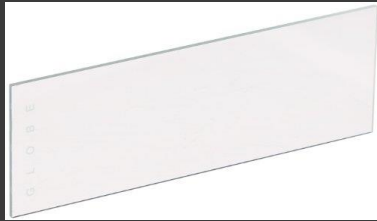


## Mikroskopie

- průchozí světlo
- fázový kontrast
- fluorescence
- konfokální
- holografická
- elektronová

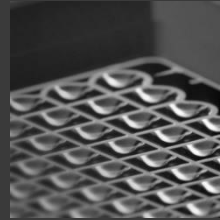


# PŘÍPRAVA A SNÍMÁNÍ



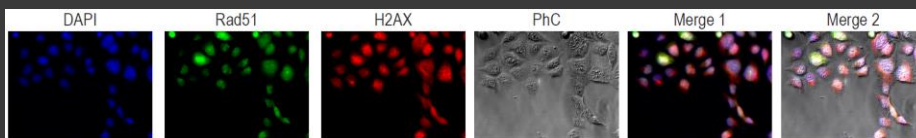
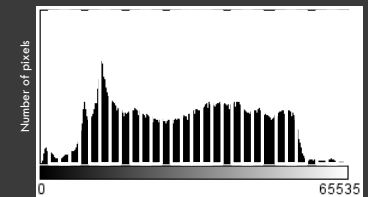
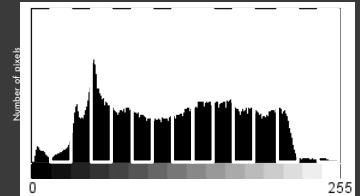
## Plast

- podložní sklo
- ztenčené dno
- více-jamkové desky



## Rozlišení

- objektiv
- bitová hloubka
- počet barev



# SNÍMÁNÍ



■ Příprava

■ Metodické

■ Automatizace

■ Reproductivita

■ Vyhodnocení

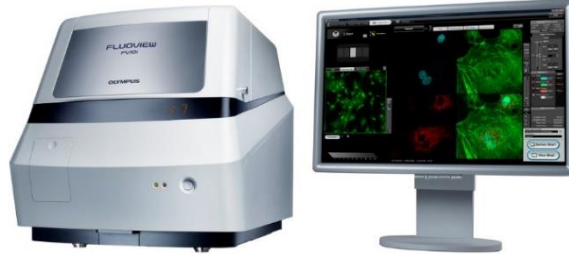
**vzorku**

# MIKROSKOP

Leica SP5/SP8



Olympus/Leica



Olympus FV10i



TissueFAXS Plus

Olympus IX70



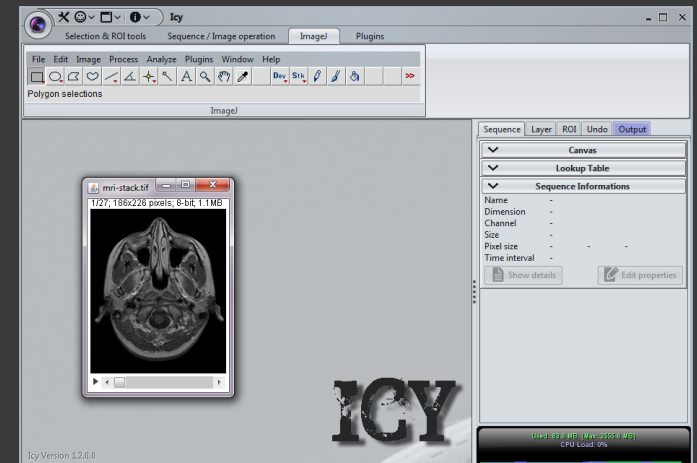
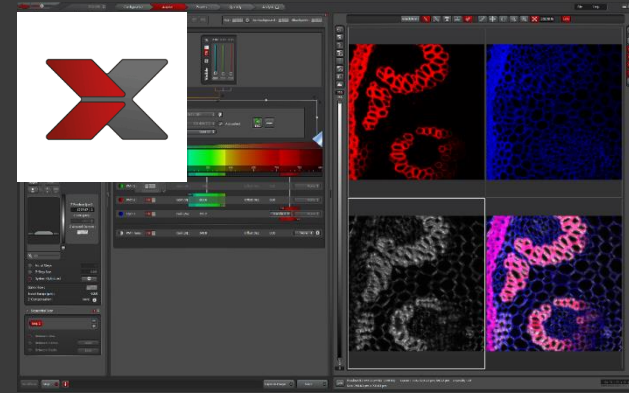
MD ImageXpress Micro

# SOFTWARE

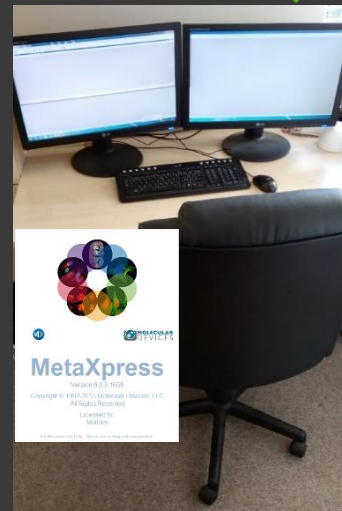


## CellProfiler

cell image analysis software



# DATA

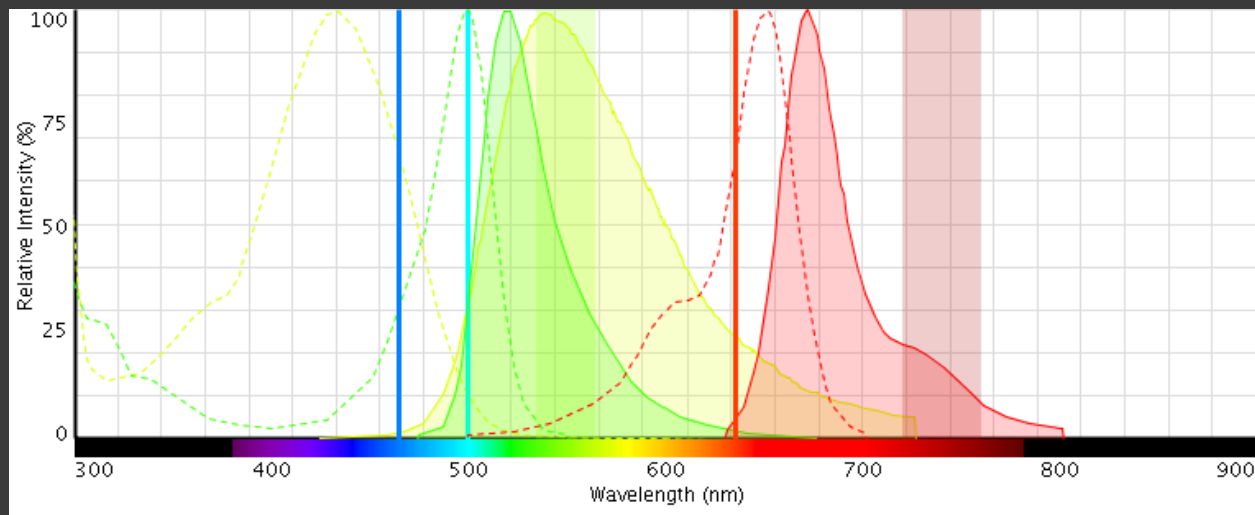
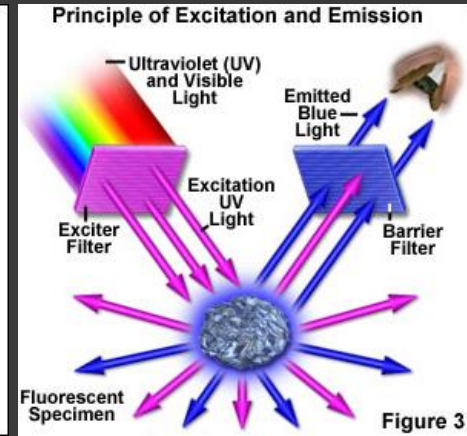
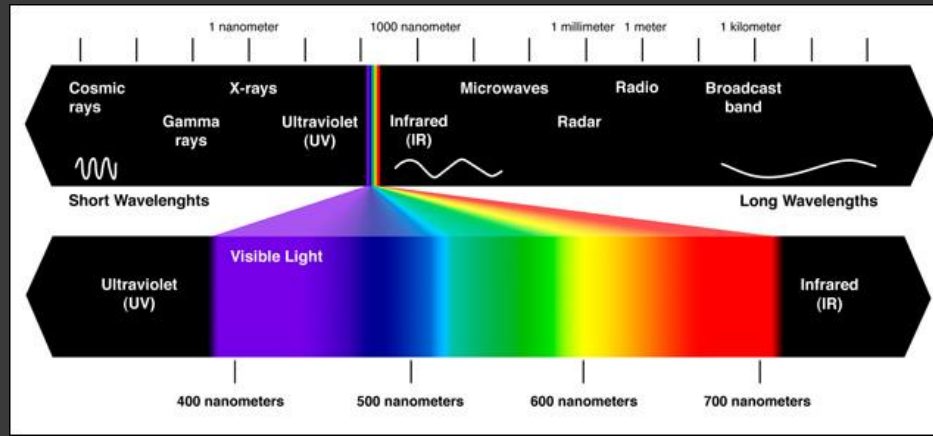
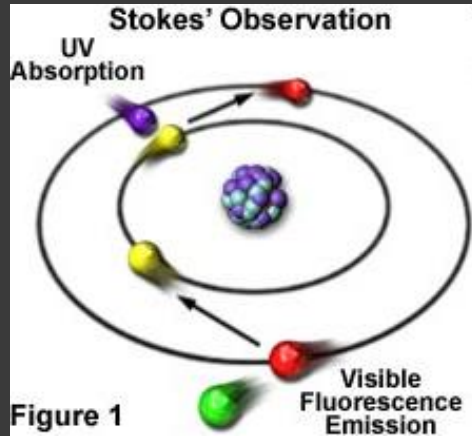


**“THE FIRST RULE OF ANY TECHNOLOGY USED IN A BUSINESS IS THAT AUTOMATION APPLIED TO AN EFFICIENT OPERATION WILL MAGNIFY THE EFFICIENCY. THE SECOND IS THAT AUTOMATION APPLIED TO AN INEFFICIENT OPERATION WILL MAGNIFY THE INEFFICIENCY.”**

Bill Gates

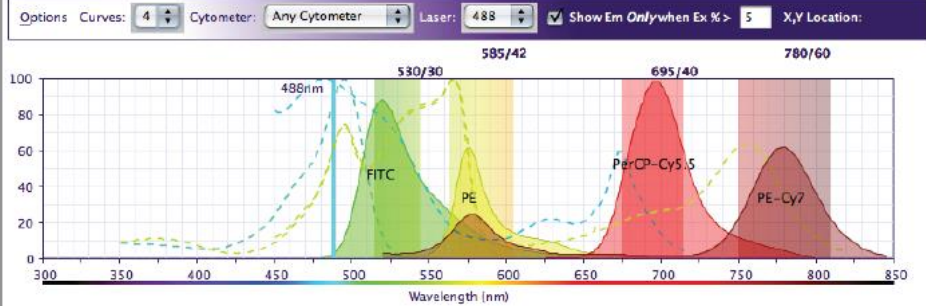


# FLUORESCENCE



# FLUOROCHROM

## BD Fluorescence Spectrum Viewer A Multicolor Tool



- Síla fluorescence fluorochromů není stejná!
- spectraviewer
- [www.fluorofinder.com](http://www.fluorofinder.com)



tips on selecting markers and criteria

+ Add a dump channel

\* Marker / Antigen      \* Target Species  Batch      Host Species      Isotype      Clone      Antigen Density

Channels 8

CD24      Human      Mouse      IgG1      SN3

+ primary/secondary

Fluorochrome Availability:

|                 |                 |                        |                      |                      |                  |
|-----------------|-----------------|------------------------|----------------------|----------------------|------------------|
| Alexa Fluor 405 | Alexa Fluor 488 | <b>Alexa Fluor 647</b> | APC                  | APC-Alexa 750        | APC-Cy7          |
| APC-eFluor 780  | APC-Vio770      | BB 515                 | Brilliant Violet 421 | Brilliant Violet 510 | DyLight 405      |
| DyLight 405LS   | DyLight 488     | ECD                    | eFluor 450           | FITC                 | Pacific Blue     |
| <b>PE</b>       | PE-Cy7          | PE-Dazzle 594          | PerCP                | PerCP-Cy5.5          | PerCP-eFluor 710 |
| PerCP-Vio700    | PE-Vio770       | VioBlue                | VioGreen             |                      |                  |

< less

CD44      Human      Mouse      IgG2a      Any

+ primary/secondary

Fluorochrome Availability:

|                        |                        |                 |            |               |         |
|------------------------|------------------------|-----------------|------------|---------------|---------|
| <b>Alexa Fluor 405</b> | <b>Alexa Fluor 488</b> | Alexa Fluor 647 | APC        | APC-Alexa 750 | APC-Cy7 |
| APC-eFluor 780         | APC-Fire 750           | APC-H7          | APC-Vio770 |               |         |

> more

Live/Dead      All Species      Any      Any      Any

+ primary/secondary

Fluorochrome Availability:

|               |               |                   |                          |               |               |
|---------------|---------------|-------------------|--------------------------|---------------|---------------|
| <b>FVS450</b> | <b>FVS510</b> | <b>FVS520</b>     | FVS570                   | <b>FVS620</b> | <b>FVS660</b> |
| <b>FVS700</b> | FVS780        | Ghost Dye Red 710 | <b>Ghost Dye Red 780</b> |               |               |

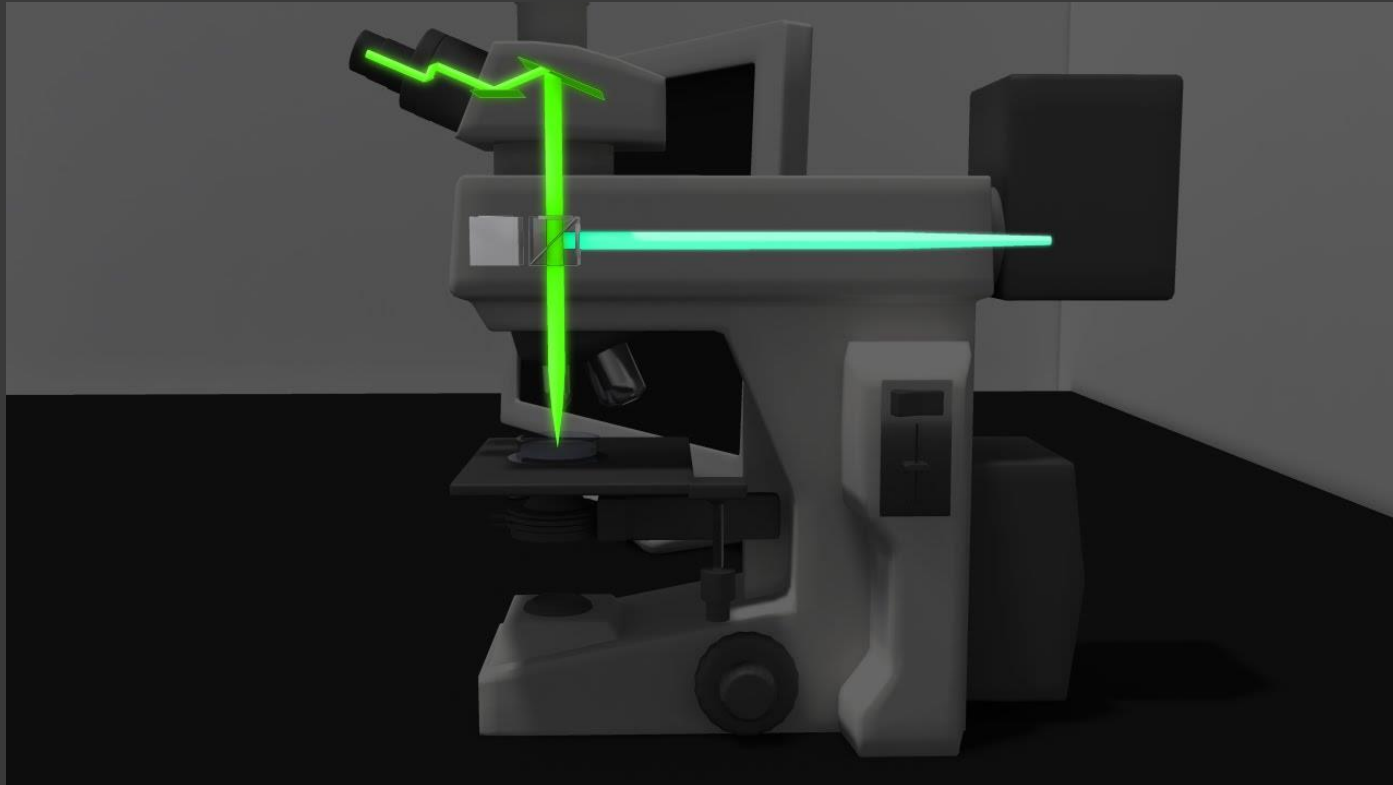
> more

# FLUOROCHROM

- Různý **JAS** fluorochromů
- Různá **stabilita/životnost** fluorochromů
- Nejméně exprimovaný protein = nejsilnější fluorochrom
- Silně exprimovaný protein = slabší fluorochrom
  
- Historicky 488nm argonový laser – FITC vs. Alexa Fluor 488 etc.

# SNÍMÁNÍ OBRAZU

- Úkolem je zachytit zvětšený obraz reality
- Čip / PMT / HyD detektor

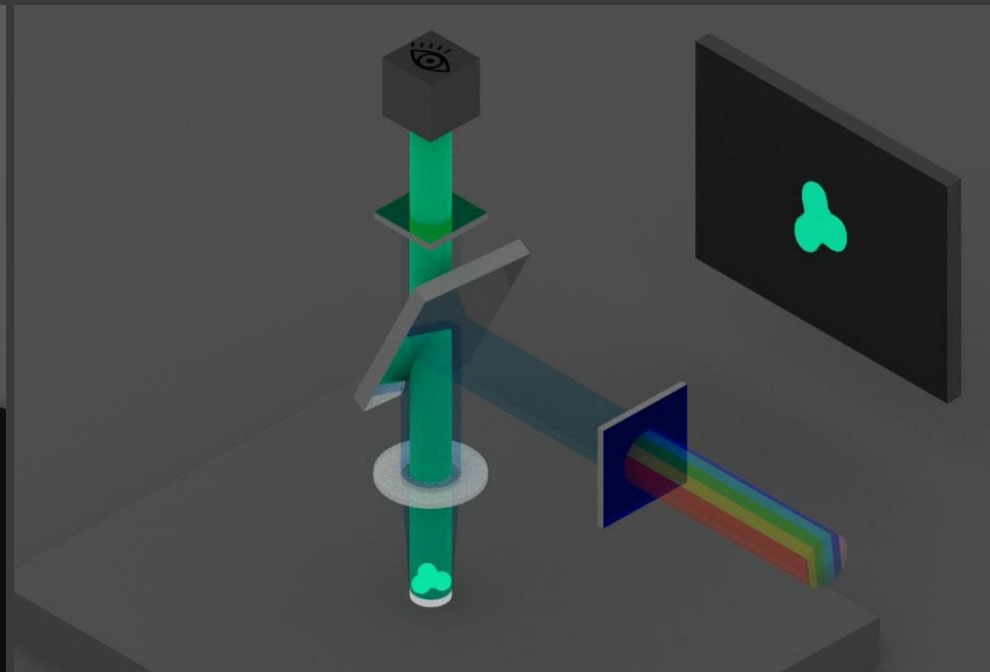
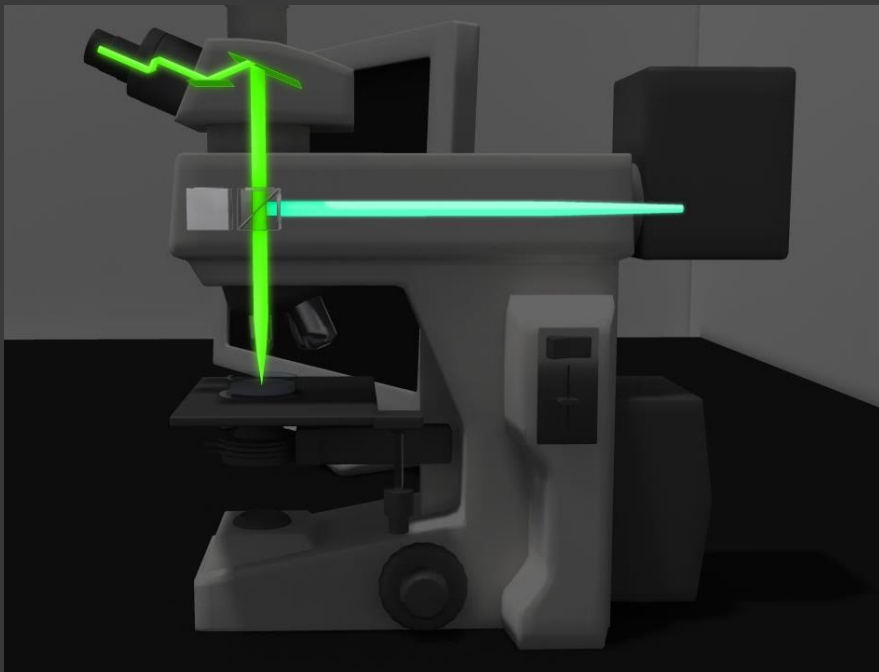
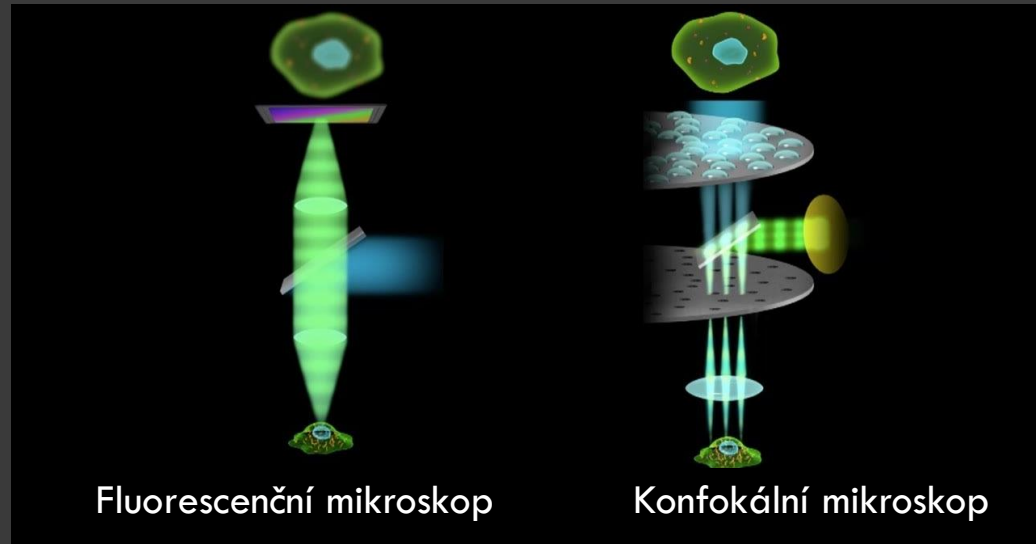


# SNÍMÁNÍ OBRAZU

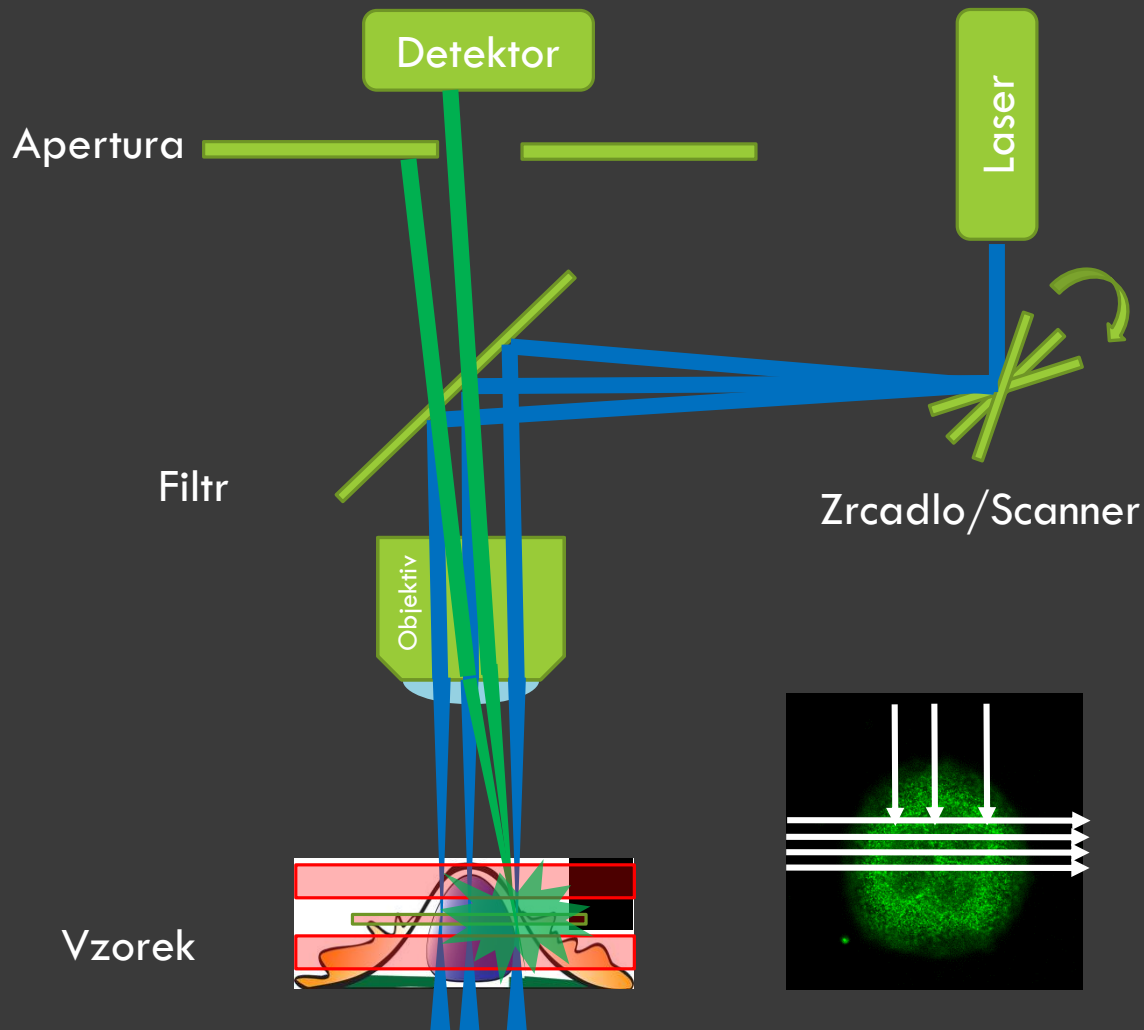
- Fluorescenční mikroskop

vs.

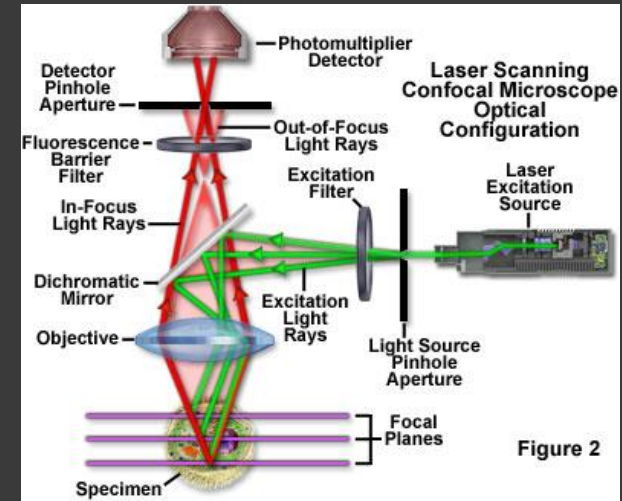
- Konfokální mikroskop



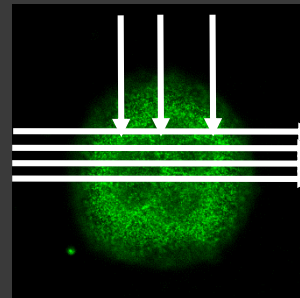
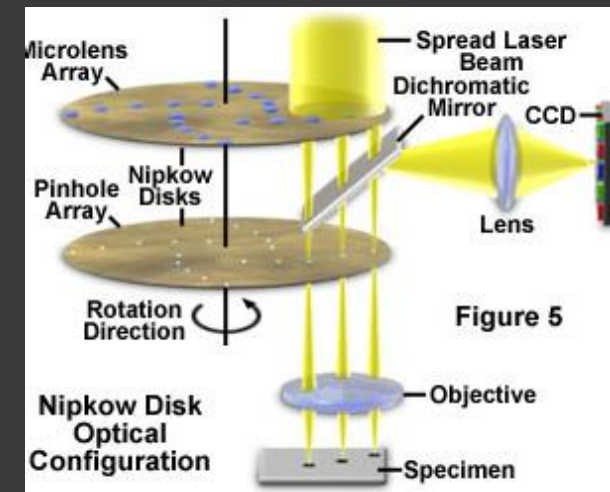
# SNÍMÁNÍ OBRAZU



## Laser scanning



## Spinning disc

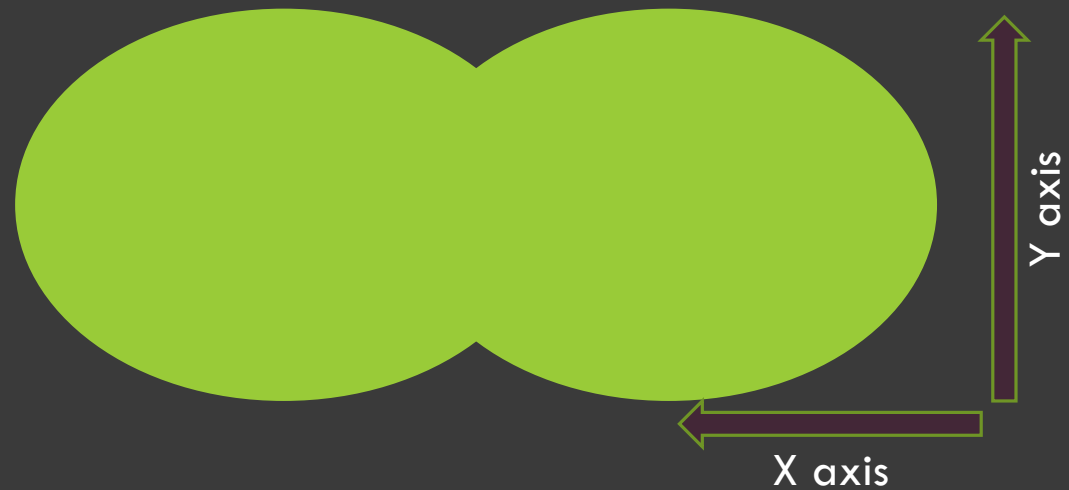
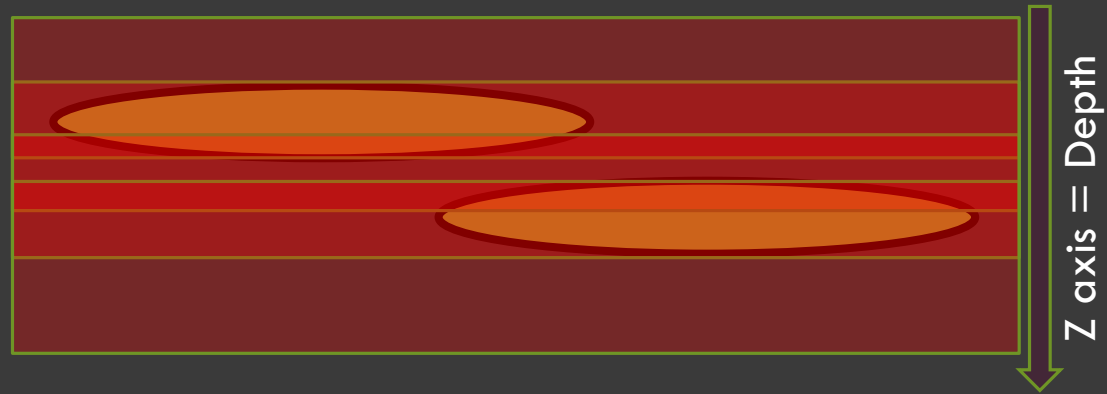
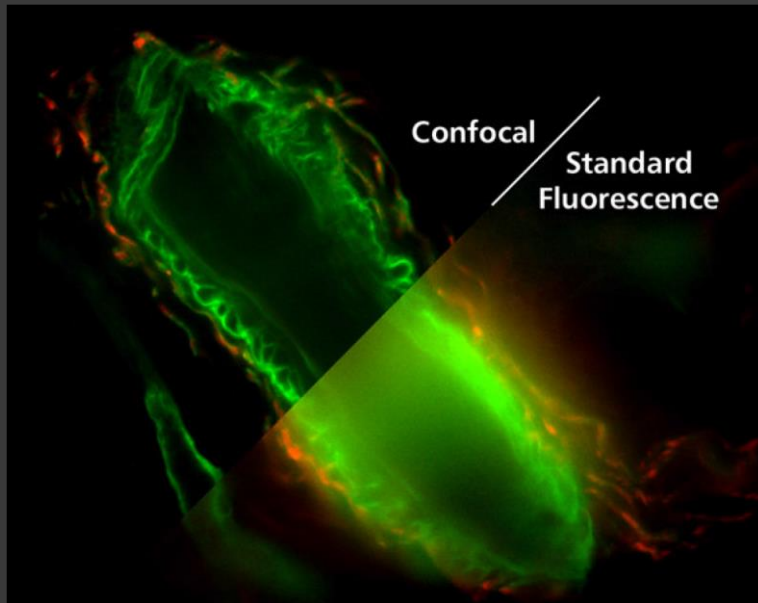


# KONFOKÁLNÍ MIKROSKOP

Leica

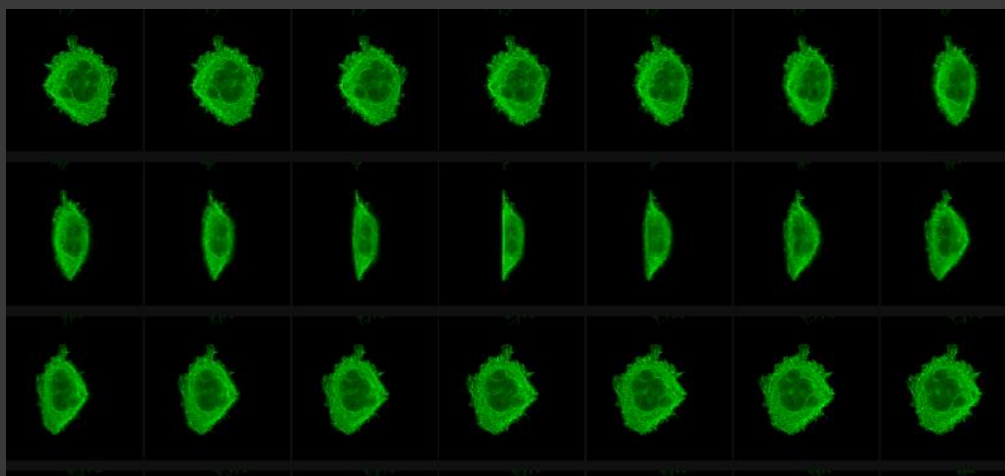
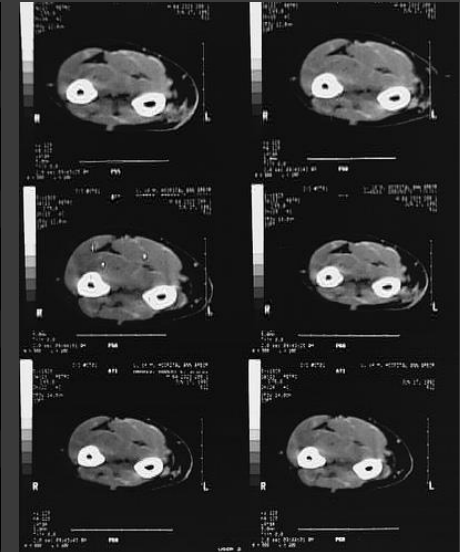
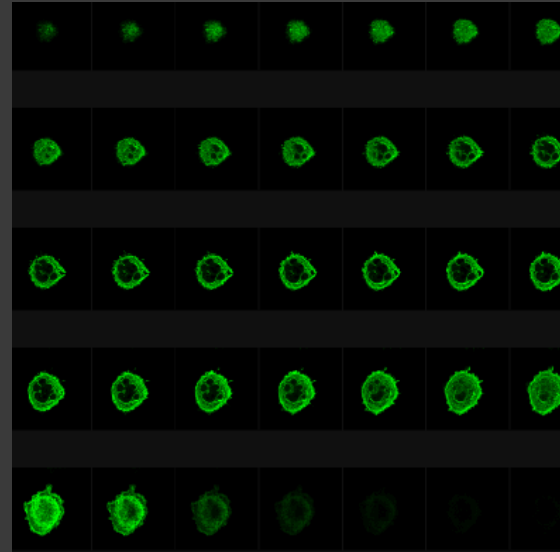


# KONFOKÁLNÍ VS. FLUORESCENČNÍ MIKROSKOP





# KONFOKÁLNÍ VS. FLUORESCENČNÍ MIKROSKOP



# LIDSKÉ OKO

- 400-700 nm (380-760)

Tyčinky (černobílé)

- 500nm, 20x více než čípků

Čípkky (R,G,B) (barevné)

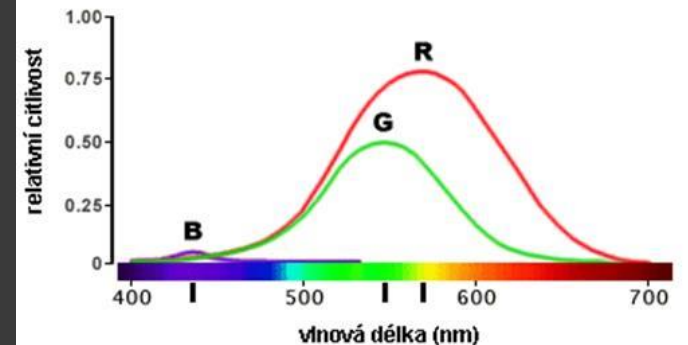
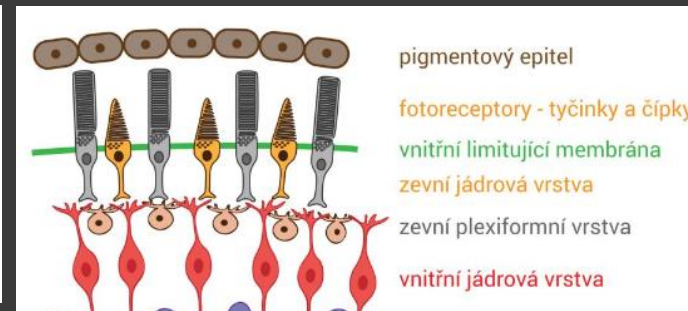
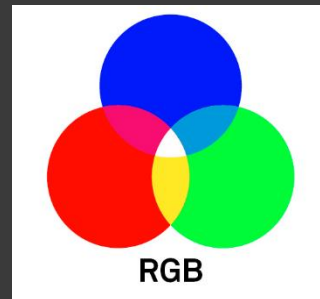
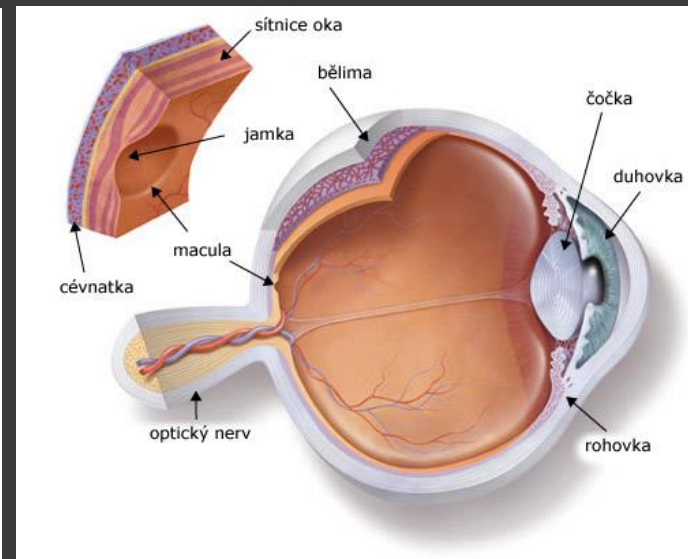
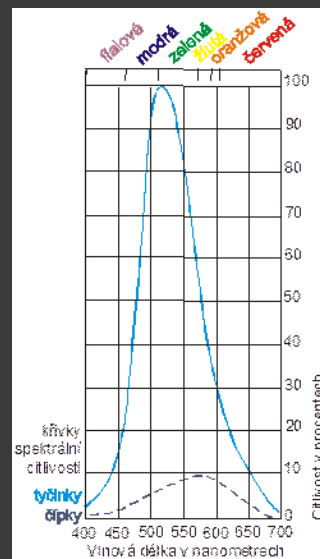
- 400-500nm Modrá – 4% 440 nm
- 500nm-600nm Zelená - 32% 540nm
- 600-700nm Červená – 64% 570 nm

- Rozlišení 0,1-0,15mm, 25 cm

- 500 stupňů šedi, 10 milionů barev

- Adaptace na tmu 40 min

- 25snímků/s pohyb, zaostření 50 věcí/s, otisk prstu 40 parametrů – duhovka 256 parametrů



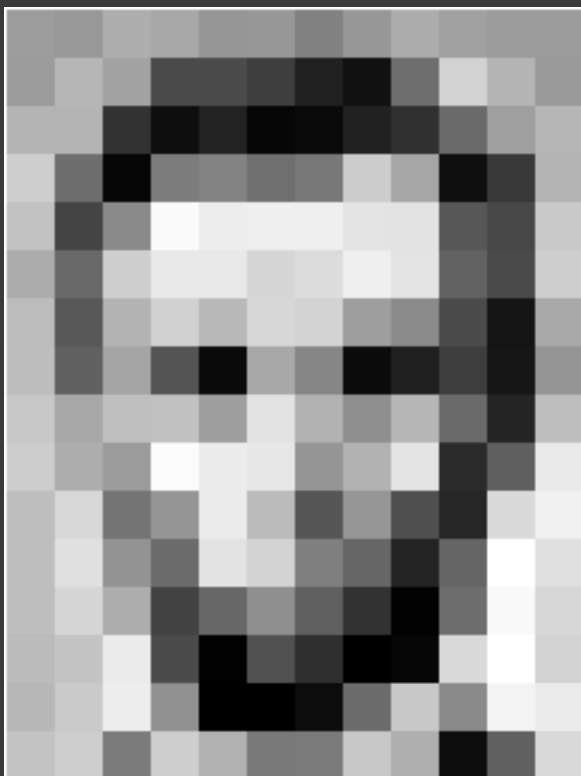

# OBRAZ

2D tabulka pixelů

COLUMNS

|   | 0   | 1   | 2   | 3   |
|---|-----|-----|-----|-----|
| 0 | 0   | 25  | 50  | 75  |
| 1 | 100 | 125 | 150 | 175 |
| 2 | 200 | 225 | 250 | 255 |

ROWS

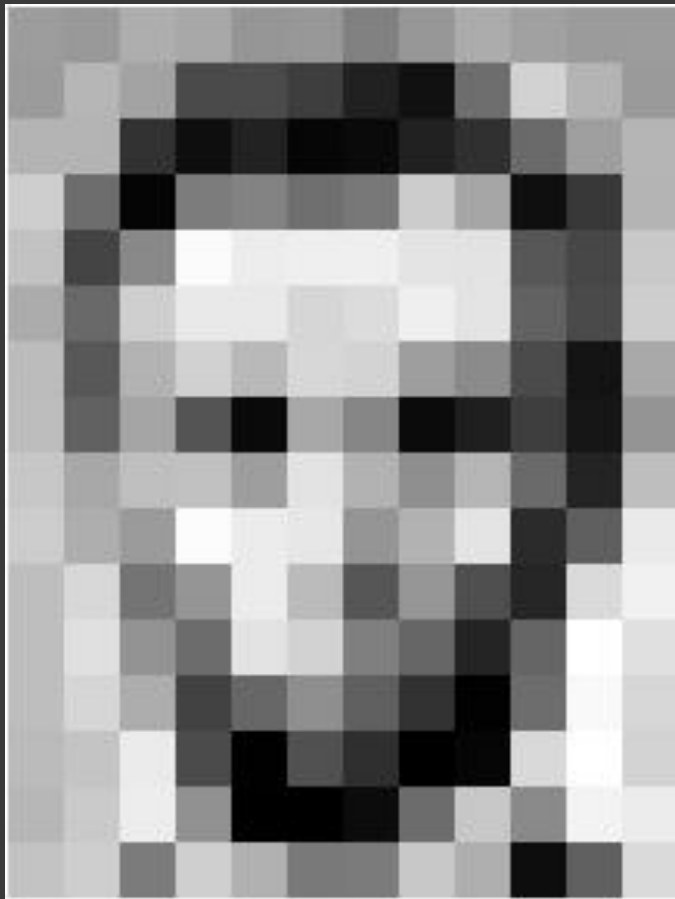
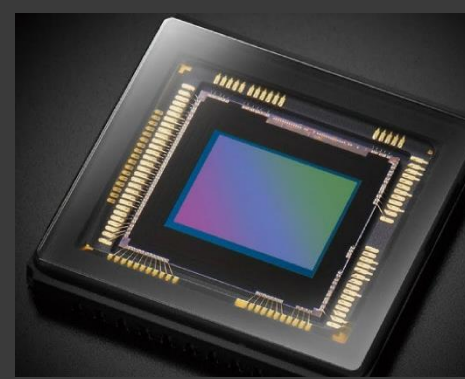


|     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 157 | 153 | 174 | 168 | 150 | 152 | 129 | 151 | 172 | 161 | 155 | 156 |
| 155 | 182 | 163 | 74  | 75  | 62  | 33  | 17  | 110 | 210 | 180 | 154 |
| 180 | 180 | 50  | 14  | 34  | 6   | 10  | 33  | 48  | 106 | 159 | 181 |
| 206 | 109 | 5   | 124 | 131 | 111 | 120 | 204 | 166 | 15  | 56  | 180 |
| 194 | 68  | 137 | 251 | 237 | 239 | 239 | 228 | 227 | 87  | 71  | 201 |
| 172 | 106 | 207 | 233 | 233 | 214 | 220 | 239 | 228 | 98  | 74  | 206 |
| 188 | 88  | 179 | 209 | 185 | 215 | 211 | 158 | 139 | 75  | 20  | 169 |
| 189 | 97  | 165 | 84  | 10  | 168 | 134 | 11  | 31  | 62  | 22  | 148 |
| 199 | 168 | 191 | 193 | 158 | 227 | 178 | 143 | 182 | 106 | 36  | 190 |
| 205 | 174 | 155 | 252 | 236 | 231 | 149 | 178 | 228 | 43  | 95  | 234 |
| 190 | 216 | 116 | 149 | 236 | 187 | 86  | 150 | 79  | 38  | 218 | 241 |
| 190 | 224 | 147 | 108 | 227 | 210 | 127 | 102 | 36  | 101 | 255 | 224 |
| 190 | 214 | 173 | 66  | 103 | 143 | 96  | 50  | 2   | 109 | 249 | 215 |
| 187 | 196 | 235 | 75  | 1   | 81  | 47  | 0   | 6   | 217 | 255 | 211 |
| 183 | 202 | 237 | 145 | 0   | 0   | 12  | 108 | 200 | 138 | 243 | 236 |
| 195 | 206 | 123 | 207 | 177 | 121 | 123 | 200 | 175 | 13  | 96  | 218 |

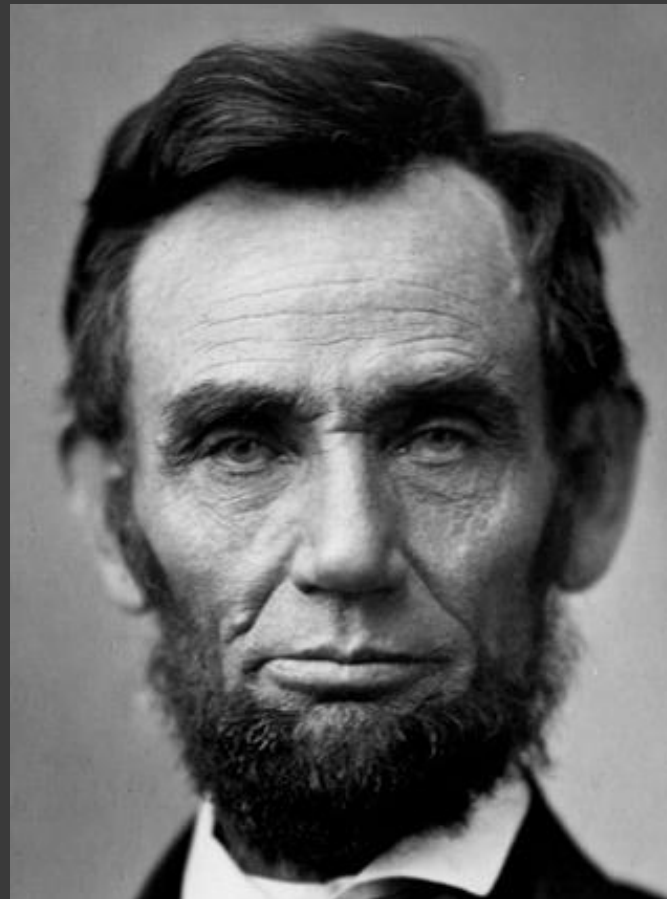
|     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 157 | 153 | 174 | 168 | 150 | 152 | 129 | 151 | 172 | 161 | 155 | 156 |
| 155 | 182 | 163 | 74  | 75  | 62  | 33  | 17  | 110 | 210 | 180 | 154 |
| 180 | 180 | 50  | 14  | 34  | 6   | 10  | 33  | 48  | 106 | 159 | 181 |
| 206 | 109 | 5   | 124 | 131 | 111 | 120 | 204 | 166 | 15  | 56  | 180 |
| 194 | 68  | 137 | 251 | 237 | 239 | 239 | 228 | 227 | 87  | 71  | 201 |
| 172 | 106 | 207 | 233 | 233 | 214 | 220 | 239 | 228 | 98  | 74  | 206 |
| 188 | 88  | 179 | 209 | 185 | 215 | 211 | 158 | 139 | 75  | 20  | 169 |
| 189 | 97  | 165 | 84  | 10  | 168 | 134 | 11  | 31  | 62  | 22  | 148 |
| 199 | 168 | 191 | 193 | 158 | 227 | 178 | 143 | 182 | 106 | 36  | 190 |
| 205 | 174 | 155 | 252 | 236 | 231 | 149 | 178 | 228 | 43  | 95  | 234 |
| 190 | 216 | 116 | 149 | 236 | 187 | 86  | 150 | 79  | 38  | 218 | 241 |
| 190 | 224 | 147 | 108 | 227 | 210 | 127 | 102 | 36  | 101 | 255 | 224 |
| 190 | 214 | 173 | 66  | 103 | 143 | 96  | 50  | 2   | 109 | 249 | 215 |
| 187 | 196 | 235 | 75  | 1   | 81  | 47  | 0   | 6   | 217 | 255 | 211 |
| 183 | 202 | 237 | 145 | 0   | 0   | 12  | 108 | 200 | 138 | 243 | 236 |
| 195 | 206 | 123 | 207 | 177 | 121 | 123 | 200 | 175 | 13  | 96  | 218 |

# ROZLIŠENÍ

- Oko: 0,1-0,15mm
- Mikroskop: 0,00017mm, Abbeho limit, Numerická apertura, zvětšení objektivu, vlnová délka



12 x 16 px



321 x 428 px

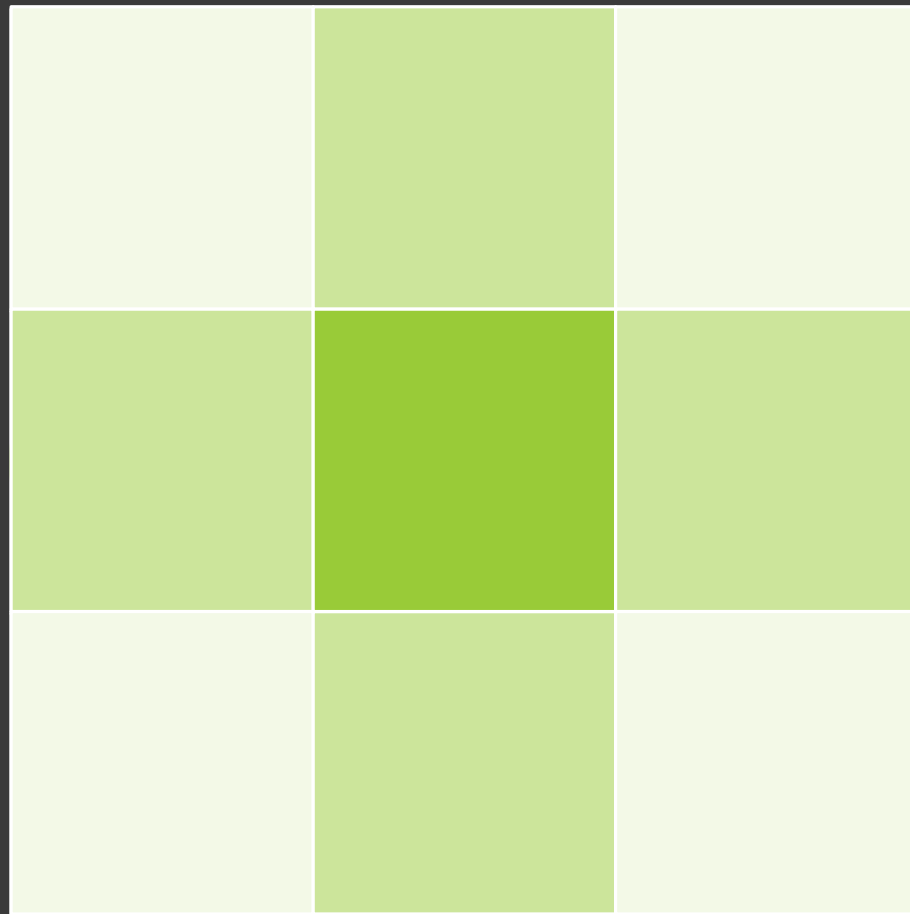
- počet pixelů
- CMOS/CCD čipy
- dpi – dots per inch
- tvar objektu vs. px
- zvětšení objektivu
- rozlišení objektivu



# ROZLIŠENÍ



1 x 1 px



3 x 3 px

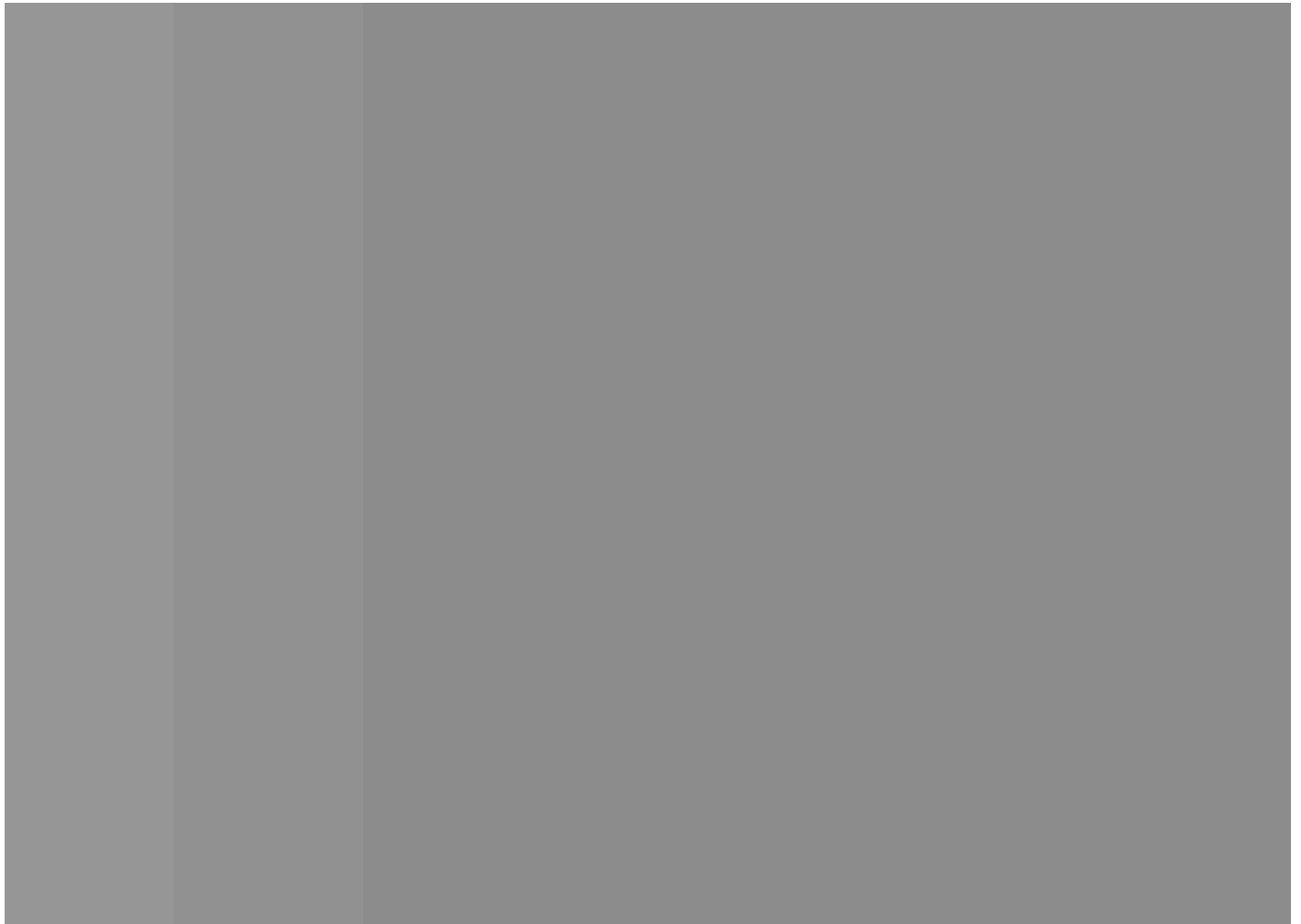
# STUPNĚ ŠEDI

- Hladiny 150, 145, 140
- 30 stupňů šedi



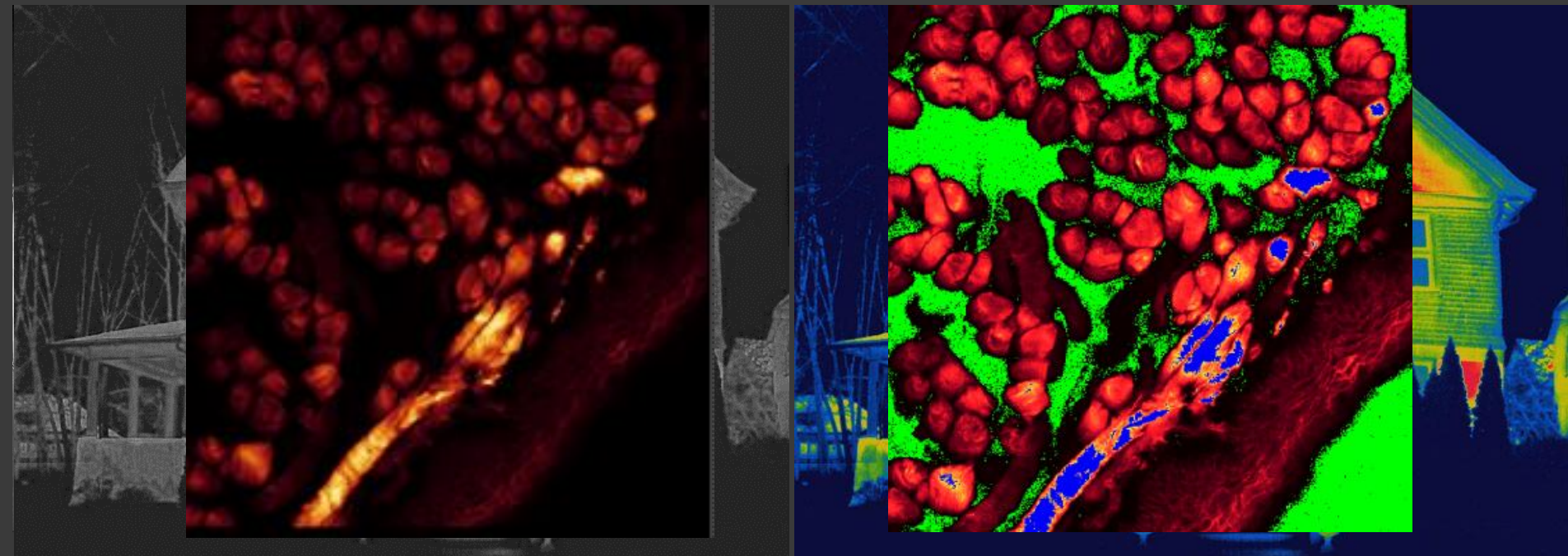
- okolo 900 stupňů šedi relativně

# STUPNĚ ŠEDI



# BAREVNÉ KÓDOVÁNÍ

- 10 milionů barev





ImageJ:  
Analyze →  
Histogram  
Image → Type

# HISTOGRAM — BITOVÁ HLOUBKA

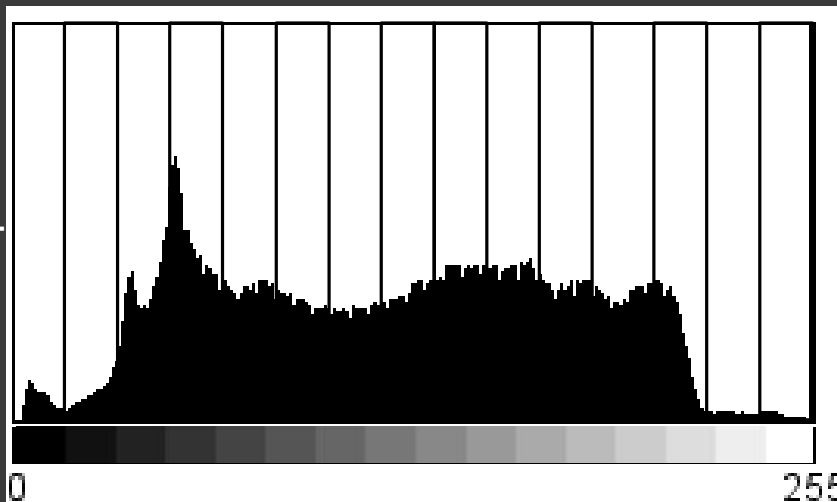
Originální obrázek

Histogram

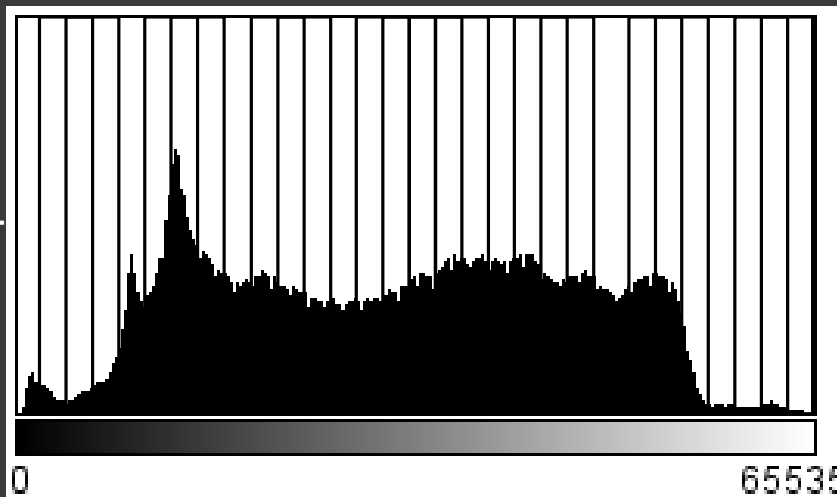
Bitová  
hloubka

8 bit

Počet pixelů



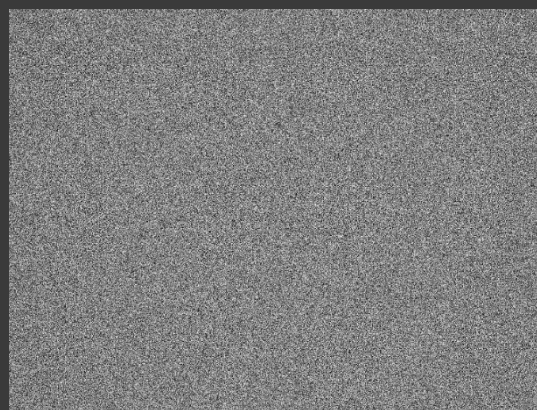
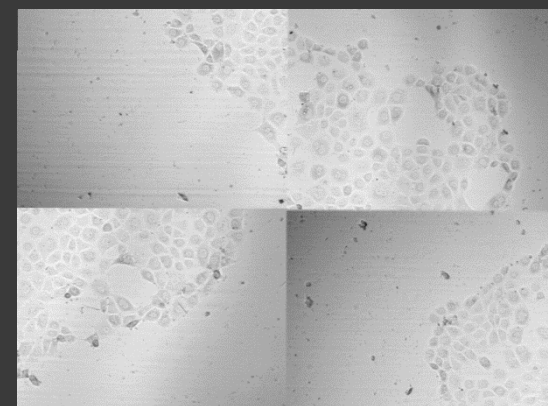
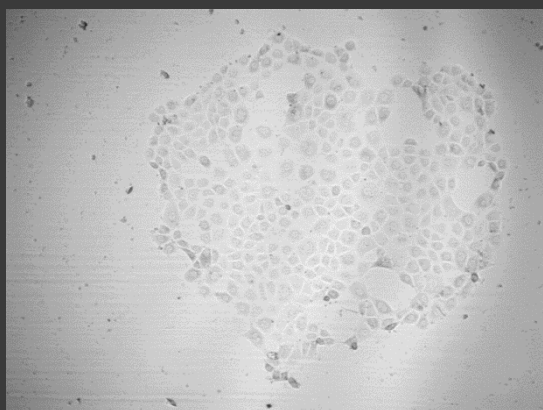
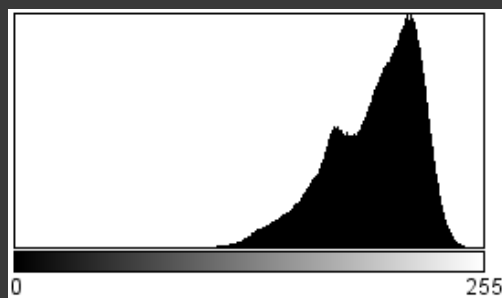
Počet pixelů



16 bit

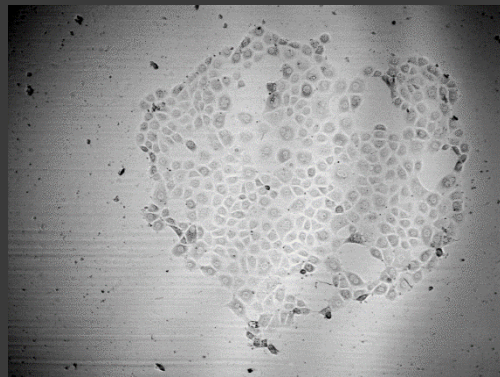
# HISTOGRAM — REKONSTRUKCE OBRAZU

Histogram

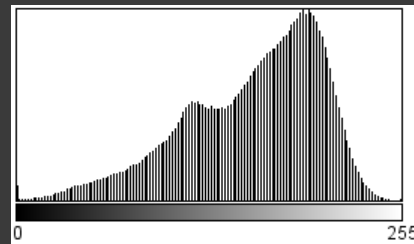


ImageJ:  
Image → Adjust →  
Brightness/Contrast

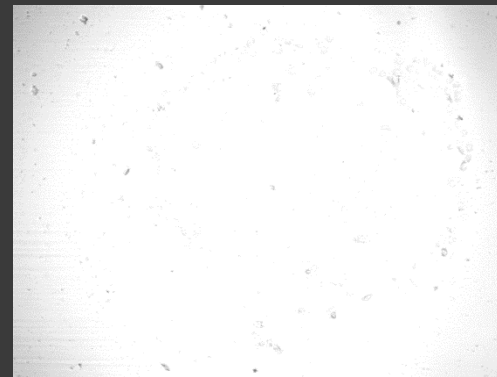
# HISTOGRAM – DYNAMICKÝ ROZSAH



Originalní histogram



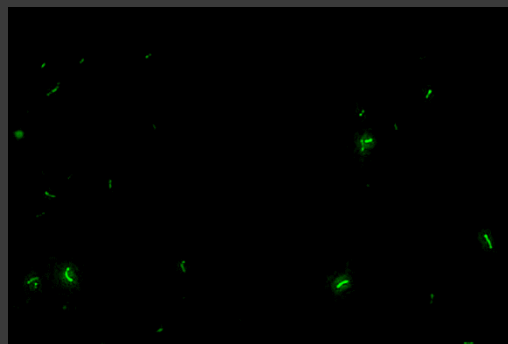
Velký dynamický rozsah



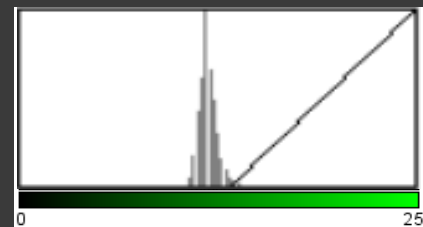
Velká expozice



Malý dynamický rozsah



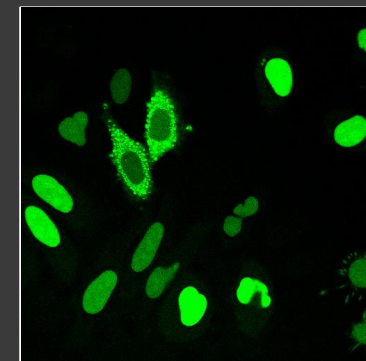
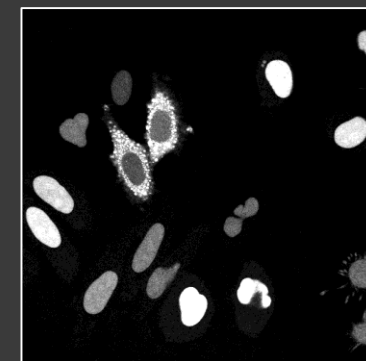
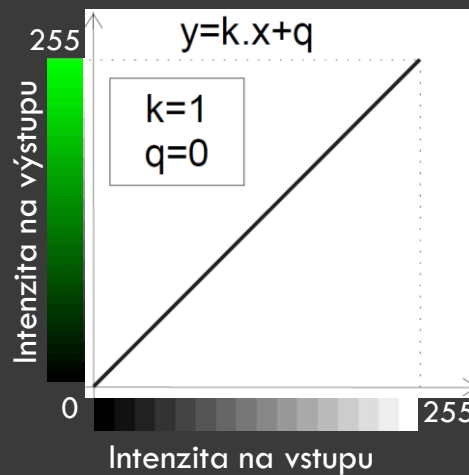
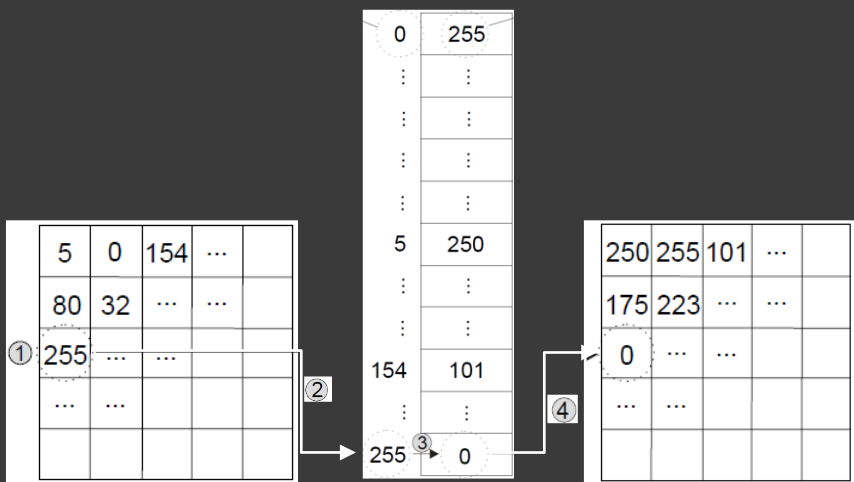
Odstředění pozadí



ImageJ:  
Image → Lookup  
Tables → Invert  
LUT/Green

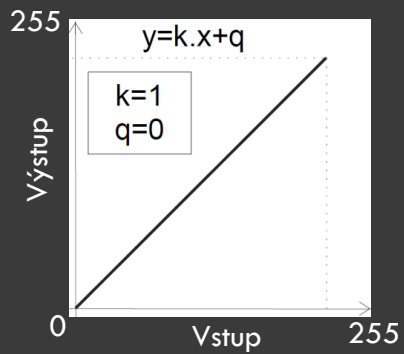
# HISTOGRAM & LUT – LOOKUP TABLE

Jak LUT funguje

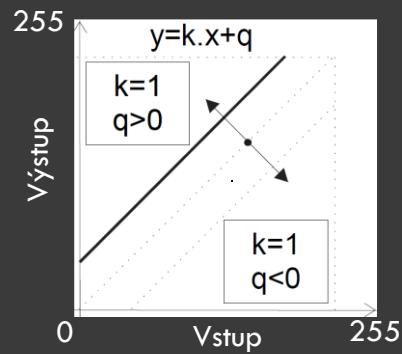


# HISTOGRAM & LUT – PŘÍKLADY

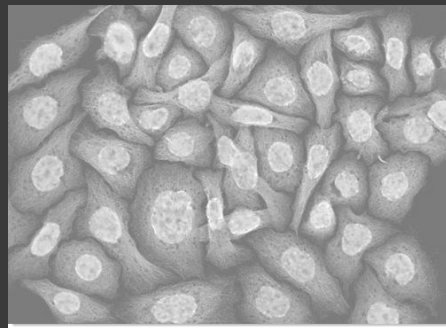
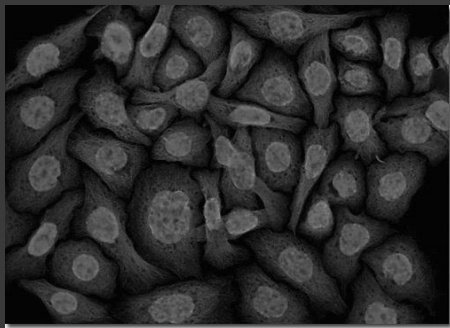
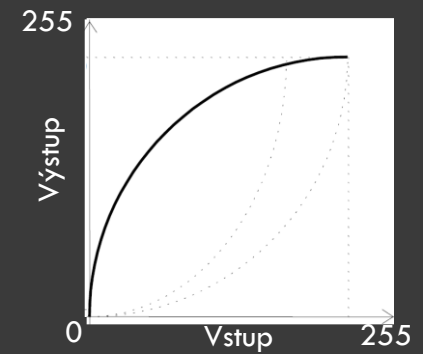
Žádná změna



Jas

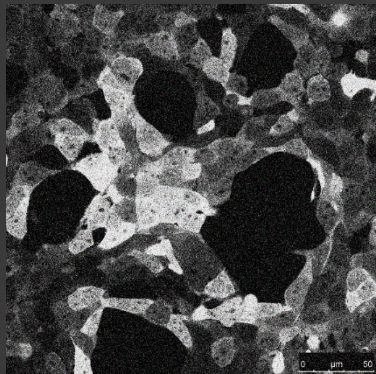


Gama korekce

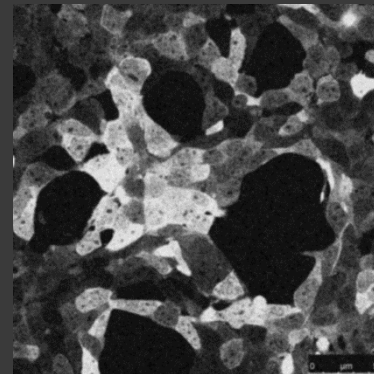


ImageJ:  
Process → Filters →  
Gaussian  
Blur/Median

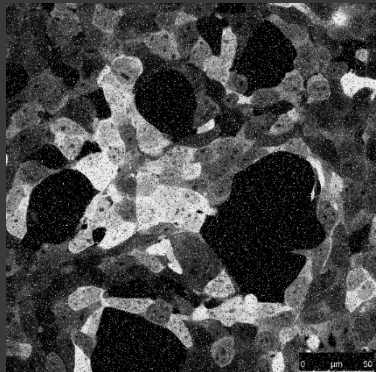
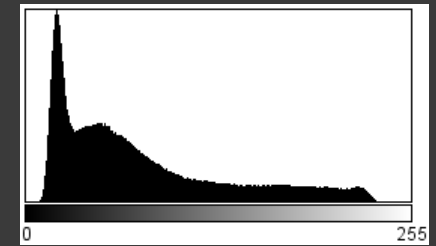
# HISTOGRAM - ŠUM



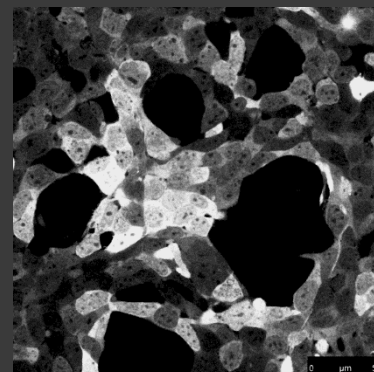
Gaussovský



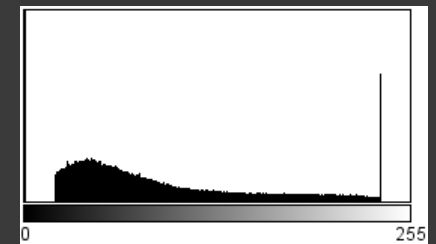
Blur filtering



Sůl & Pepř



Median filtering



# DATOVÉ FORMÁTY

## Rastrové obrazy

Tvar reprezentovaný maticí pixelů

Pixelace

Fotorealistické obrazy

Typické formáty: JPEG, GIF, TIFF, PNG

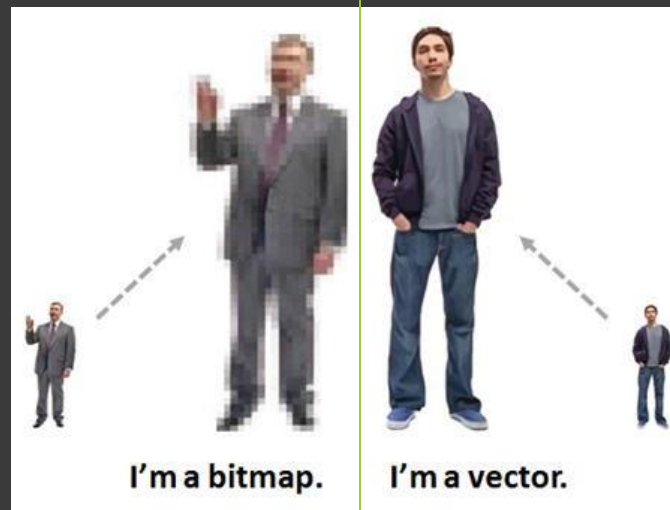
## Vektorové obrazy

Tvar složený z geometrických objektů

Jemná struktura

Diagramy, schémata, grafy, ilustrace

Typické formáty: SVG, DWG, CDR, WMF



# DATOVÉ FORMÁTY – RASTROVÁ GRAFIKA

## Portable Network Graphics (PNG)

Grafika, obrazy s textem, fotky

Bezeztrátová komprese

Velké oblasti sytých barev, ostré přechody

Nepodporuje Non-RGB barevný prostor(CMYK)

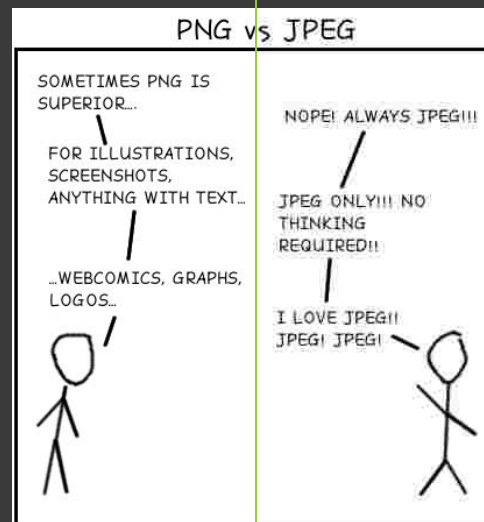
## Joint Photographic Expert Group (JPEG)

Fotky a realistické obrazy

Ztrátová komprese

Jemné, pozvolné přechody

Nepodporuje transparentnost





# DATOVÉ FORMÁTY – RASTROVÁ GRAFIKA



## Joint Photographic Expert Group (JPEG)

Fotky a realistické obrazy

Ztrátová komprese

Jemné, pozvolné přechody

Nepodporuje transparentnost

Rozdělení na bloky 8x8 pixelů, 2D DCT

Lidský zrak je citlivý k relativně malé změně v jasu nebo v barvě na poměrně velké ploše. V rozlišování konkrétní síly rychle se měnícího jasu (vysokofrekvenční změny) je však mnohem horší.



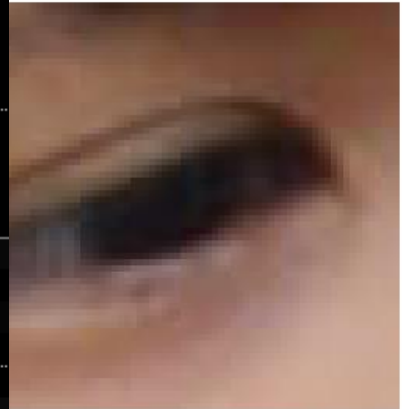
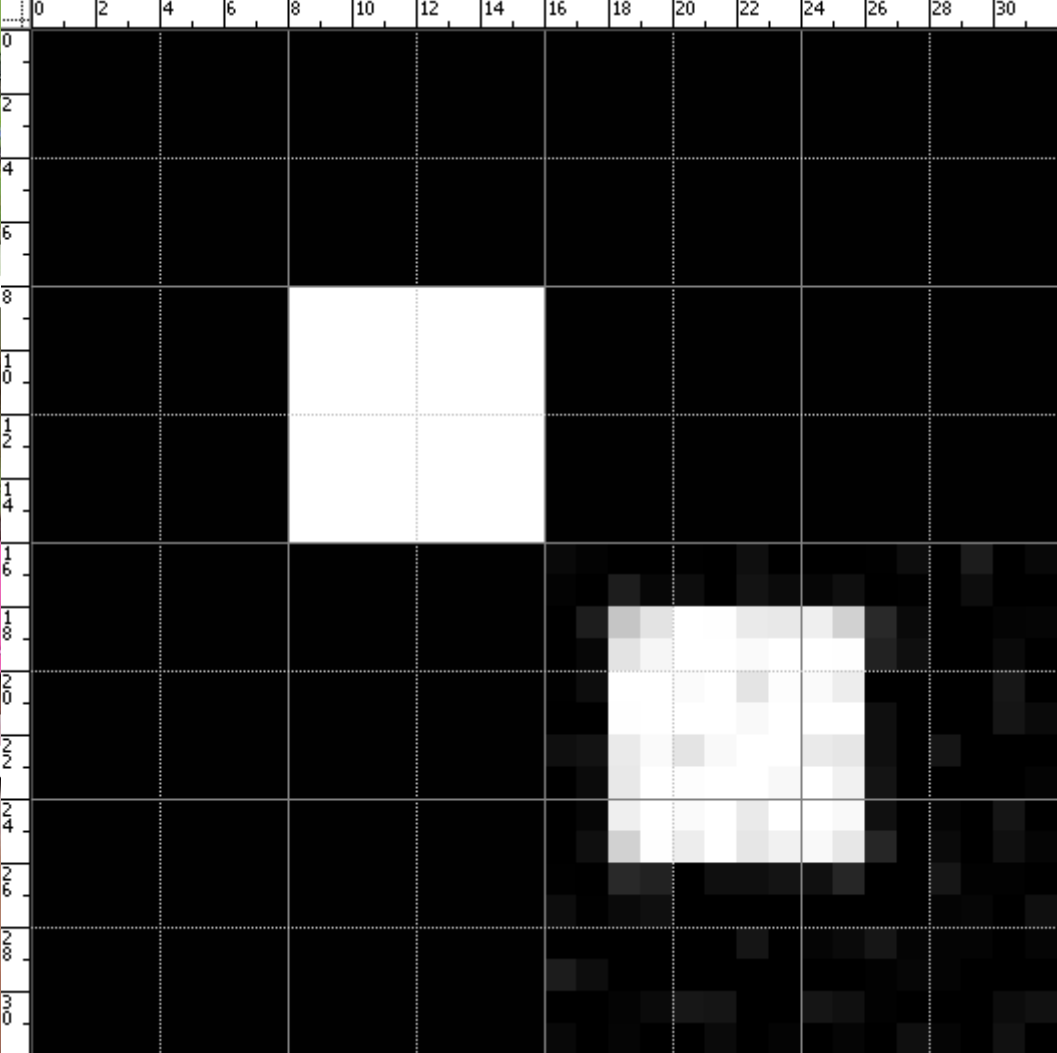
6,25% jpeg compression 0/12



400% jpeg compression 0/12



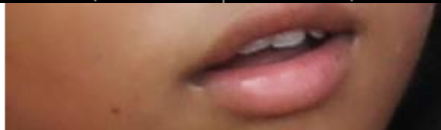
25% jpeg compression 3/12



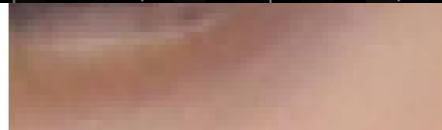
400% jpeg compression 6/12



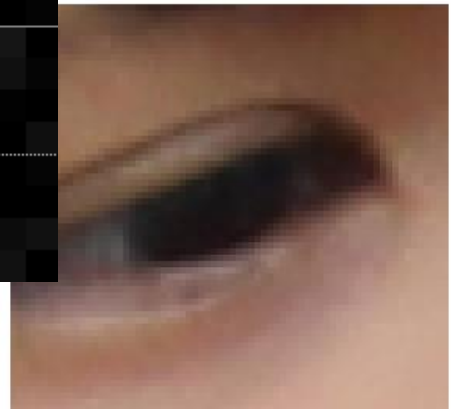
100% jpeg compression 9/12



100% jpeg compression 12/12



400% jpeg compression 12/12



400% no compression (original)

ImageJ:  
File → Save As →  
Gif/Animated  
Gif/Tiff/Compressed  
TIFF

# DATOVÉ FORMÁTY – RASTROVÁ GRAFIKA

## Graphic Interchange Format (GIF)

Grafika, loga, animace

Bezeztrátová komprese

Velké oblasti sytých barev, ostré přechody

8bits/pixel, 256 barev z 24bit RGB prostoru



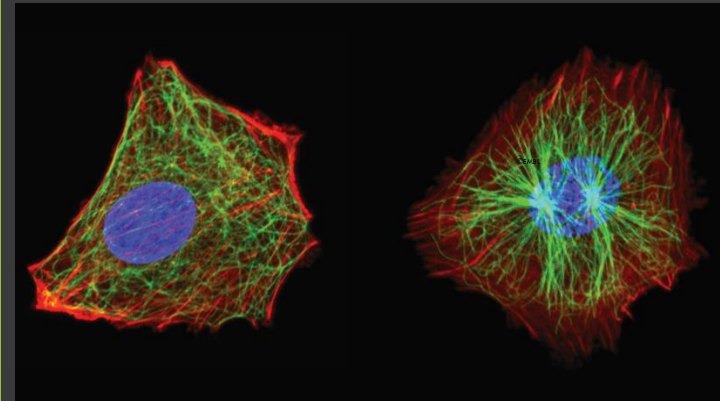
## Tagged Image File Format (TIFF)

Fotky, grafika

Možnost bezzeztrátové komprese

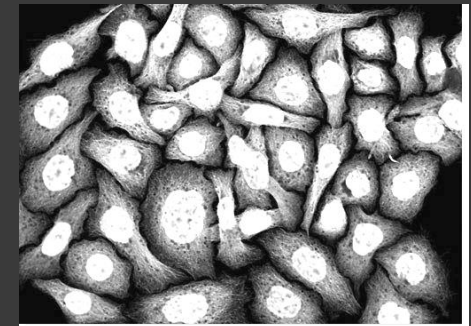
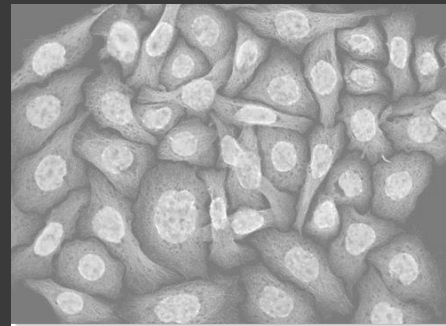
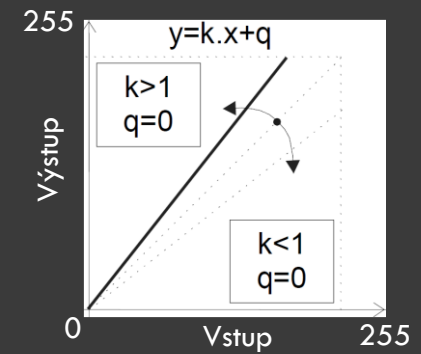
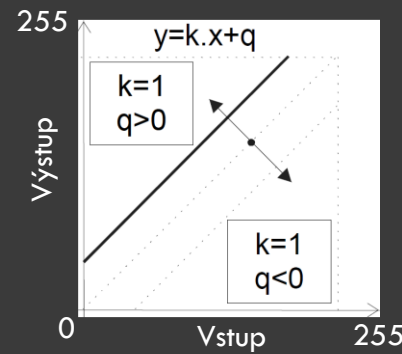
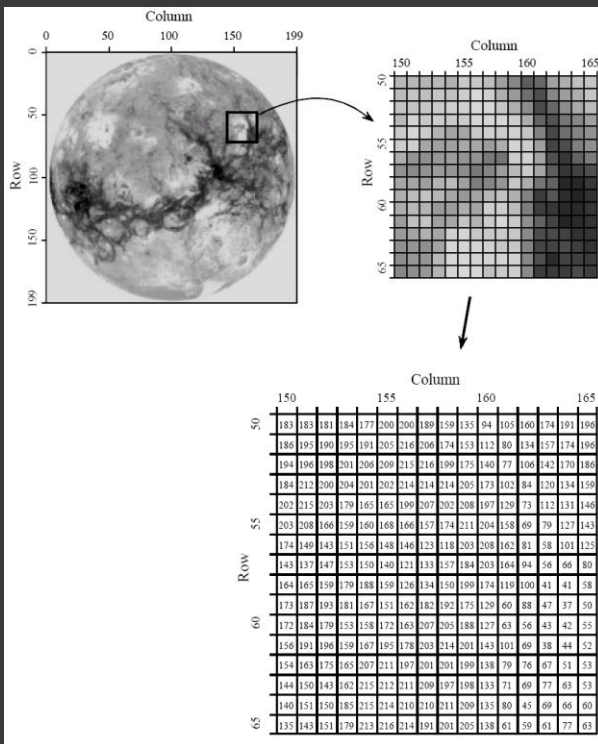
Profesionální, široce podporovaný

Různé barevné prostory, jakékoli rozlišení a barvy



ImageJ:  
Image → Adjust →  
Brightness/Contrast

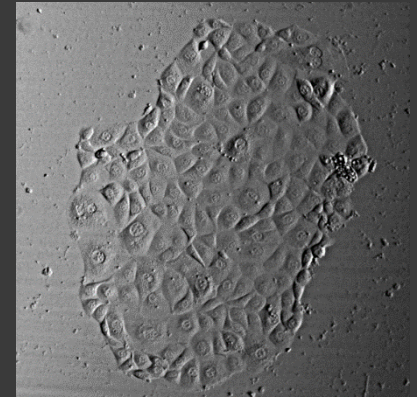
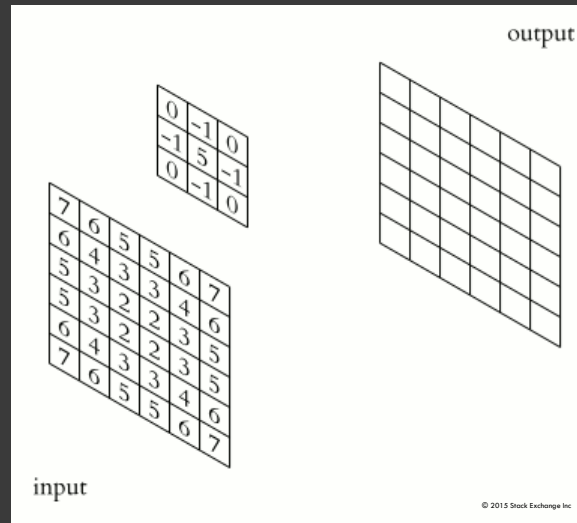
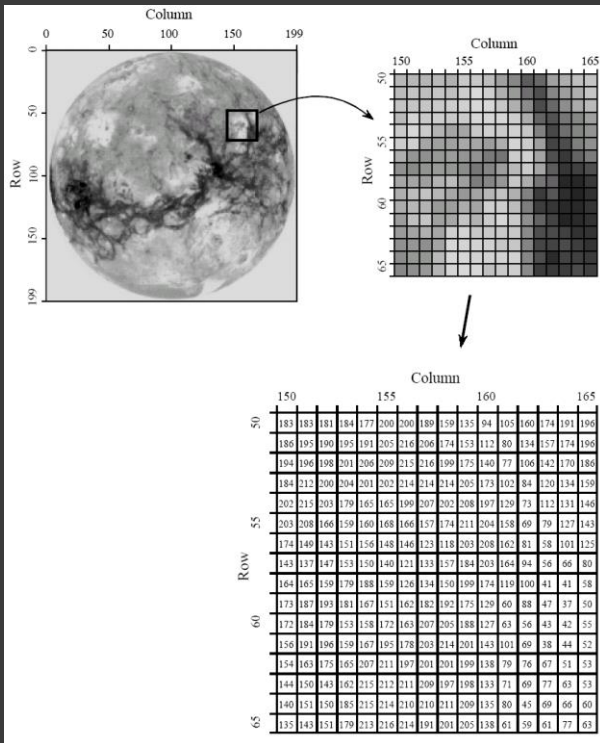
# ZPRACOVÁNÍ – BODOVÉ TRANSFORMACE



\* 30

ImageJ:  
 Process →  
 Sharpen/Filters →  
 Unsharp Mask

# FILTROVÁNÍ – LOKÁLNÍ ZMĚNY



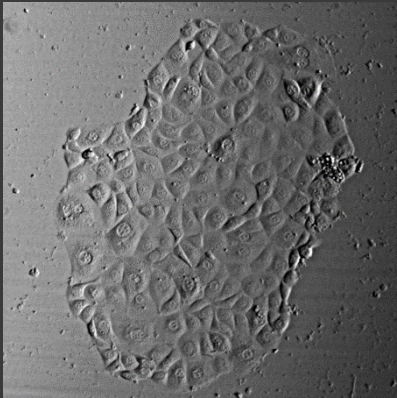
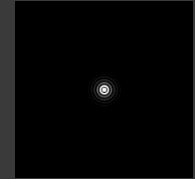
ImageJ:  
Process →  
Sharpen/Smooth/  
Find Edges  
Plugins → Parallel  
Iterative Deconvolution

# FILTROVÁNÍ – PŘÍKLADY

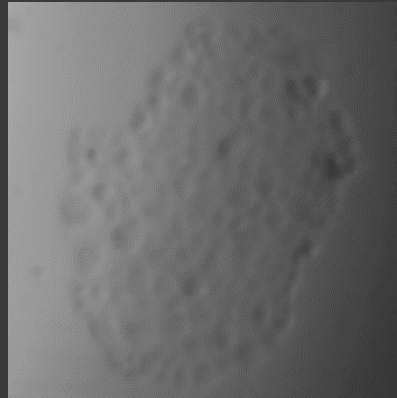
|   |    |    |    |   |
|---|----|----|----|---|
| 0 | 0  | 0  | 0  | 0 |
| 0 | 0  | -1 | 0  | 0 |
| 0 | -1 | 5  | -1 | 0 |
| 0 | 0  | -1 | 0  | 0 |
| 0 | 0  | 0  | 0  | 0 |

|   |   |   |   |   |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 0 | 1 | 1 | 1 | 0 |
| 0 | 1 | 1 | 1 | 0 |
| 0 | 1 | 1 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 |

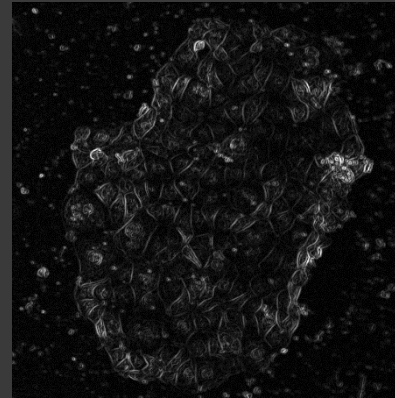
|  |   |    |   |  |
|--|---|----|---|--|
|  |   |    |   |  |
|  | 0 | 1  | 0 |  |
|  | 1 | -4 | 1 |  |
|  | 0 | 1  | 0 |  |
|  |   |    |   |  |



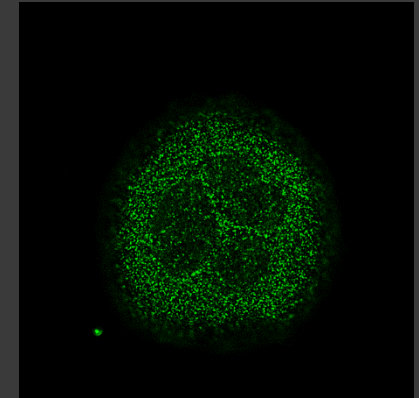
doostření



rozmazání



detekce hran

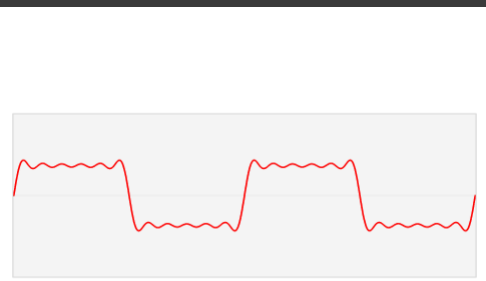
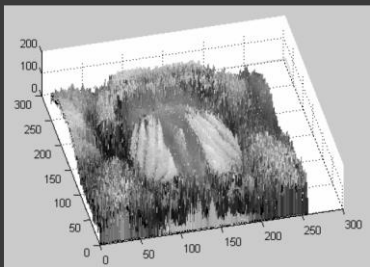
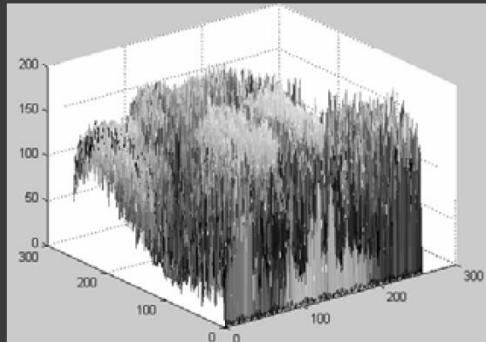
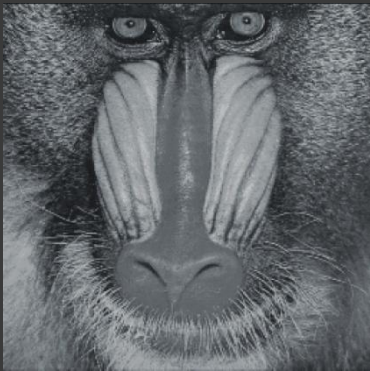


dekonvoluce

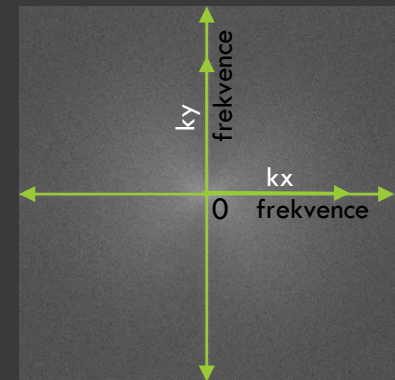
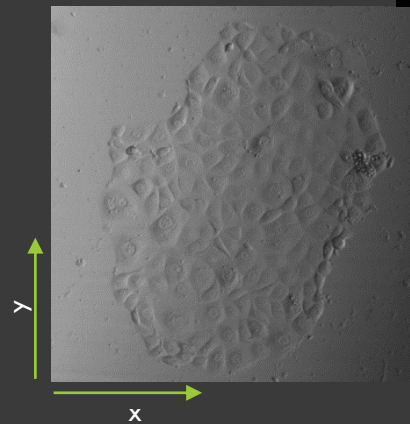
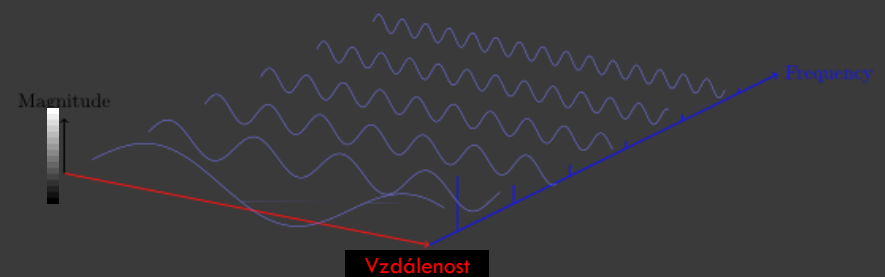
ImageJ:  
Process → FFT →  
FFT

# FILTROVÁNÍ – GLOBÁLNÍ TRANSFORMACE

## Fast Fourier Transformation

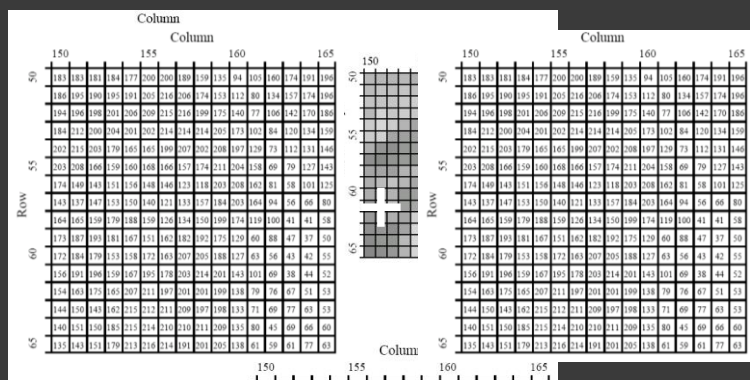


Konvoluce = Násobení

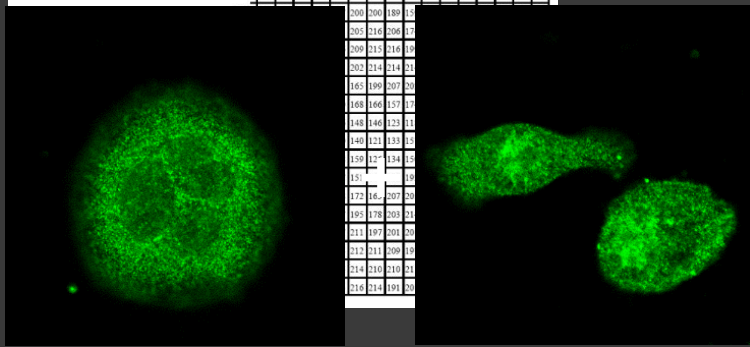


# OBRAZOVÁ ARITMETIKA

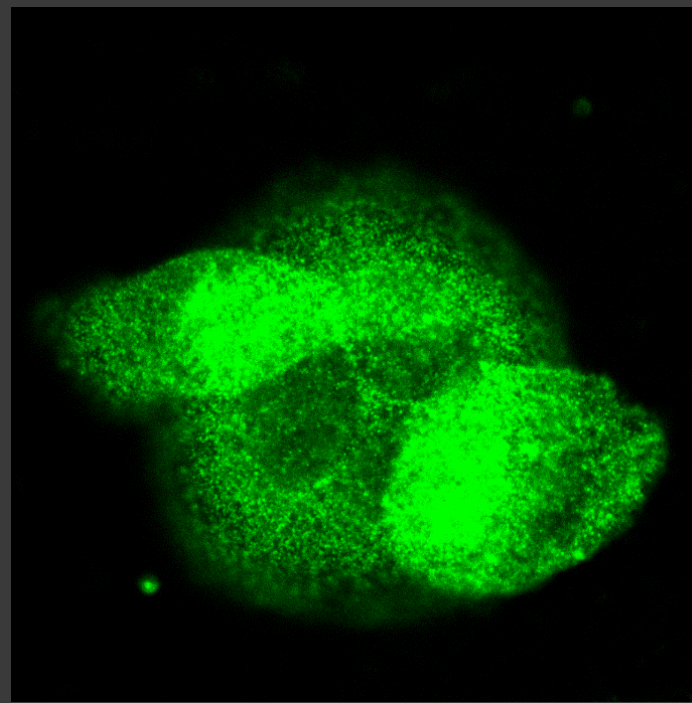
## Sčítání



==



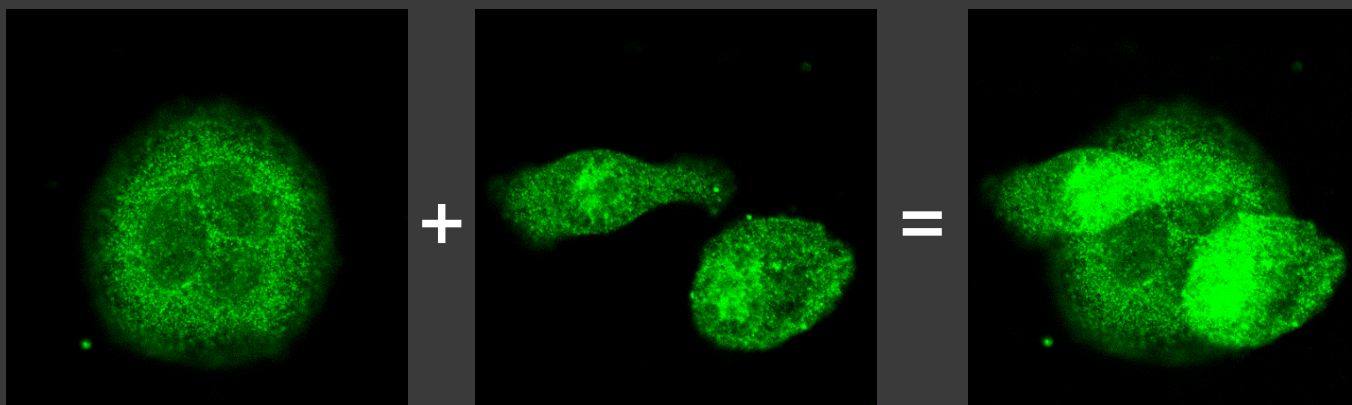
==



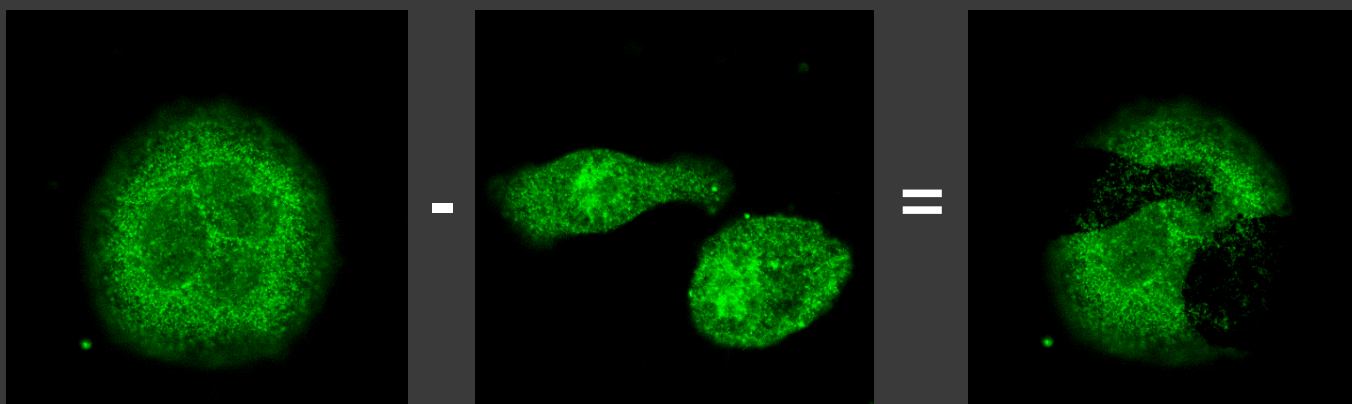


# OBRAZOVÁ ARITMETIKA

Sčítání

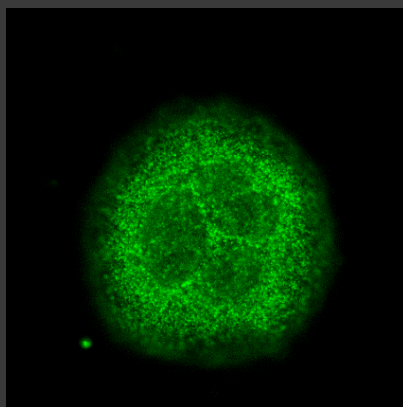


Odčítání

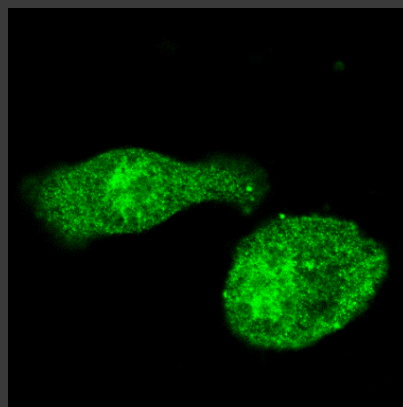


# OBRAZOVÁ ARITMETIKA

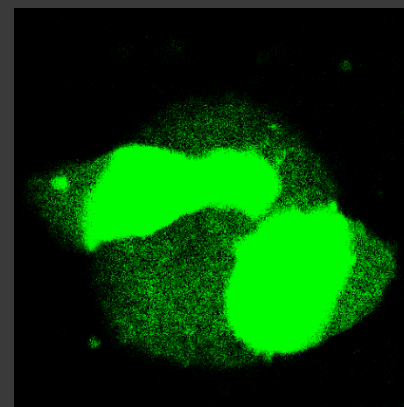
Násobení



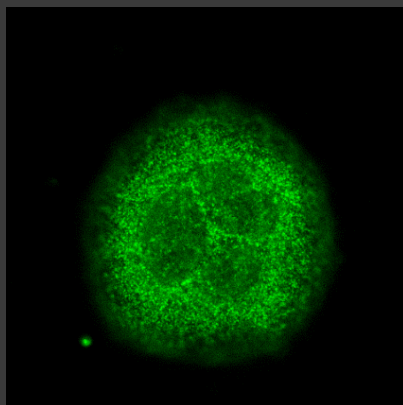
x



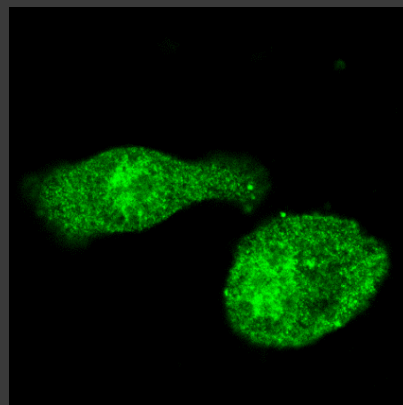
=



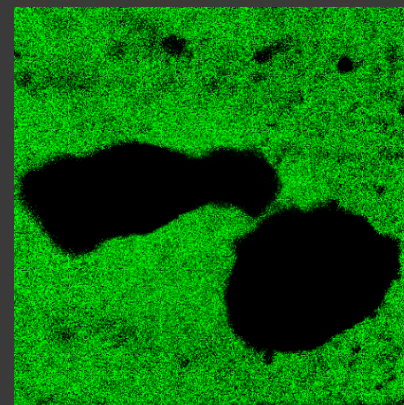
Dělení



/

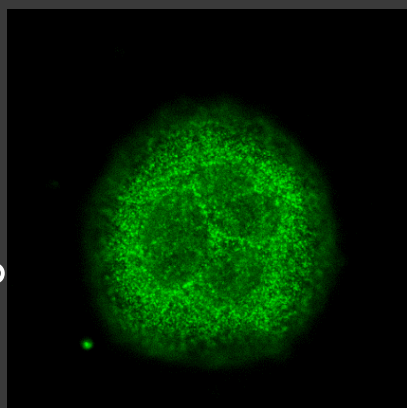


=

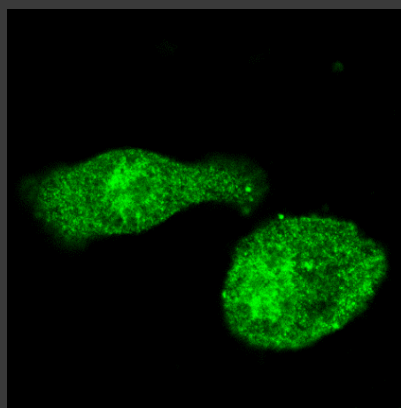


# OBRAZOVÁ ARITMETIKA

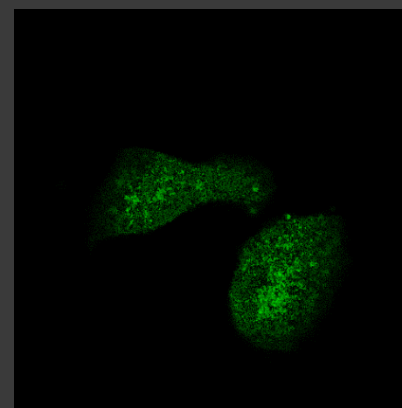
Logické AND



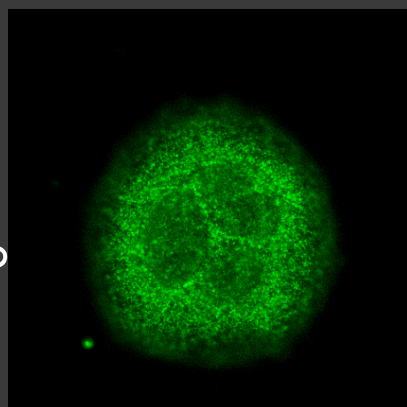
AND



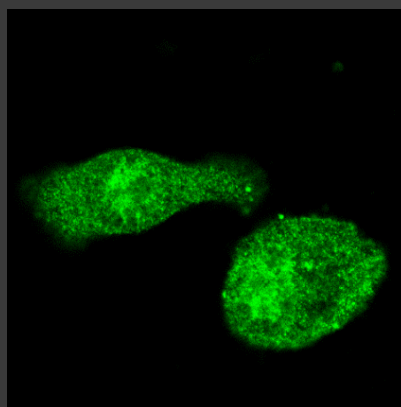
==



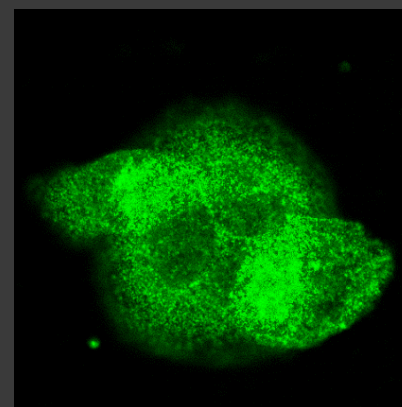
Logické OR



OR



==



ImageJ:

Process → Filters →

Gaussian Blur →

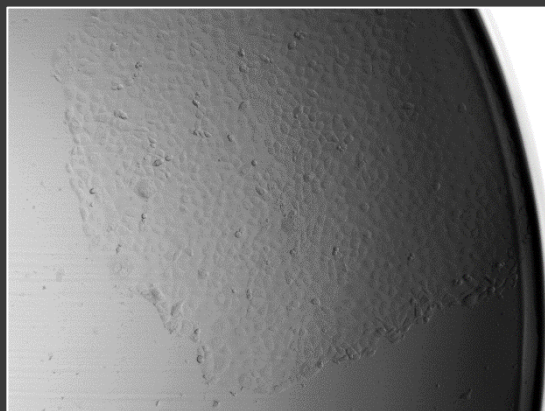
Save As

Process → Image

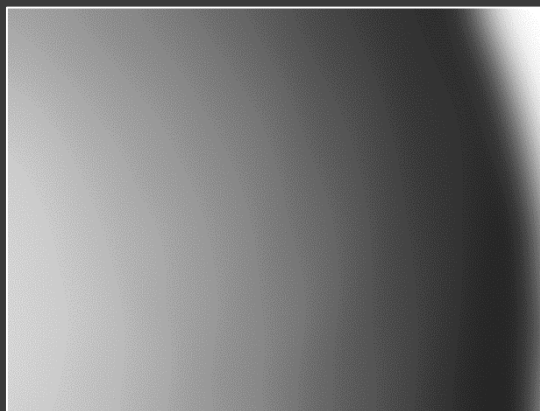
Calculator

# OBRAZOVÁ ARITMETIKA – PŘÍKLADY

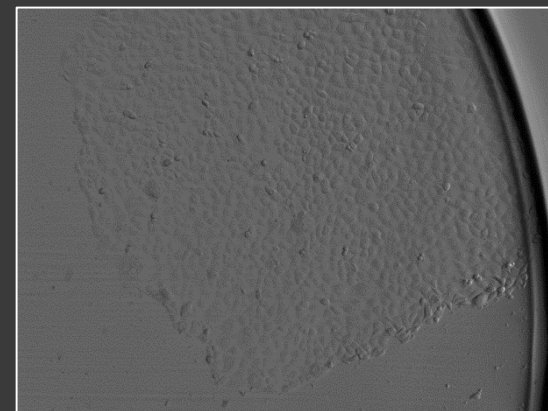
Flat Field korekce



/



=



Gaussian blur

ImageJ:

Image → Adjust →

Threshold → Save

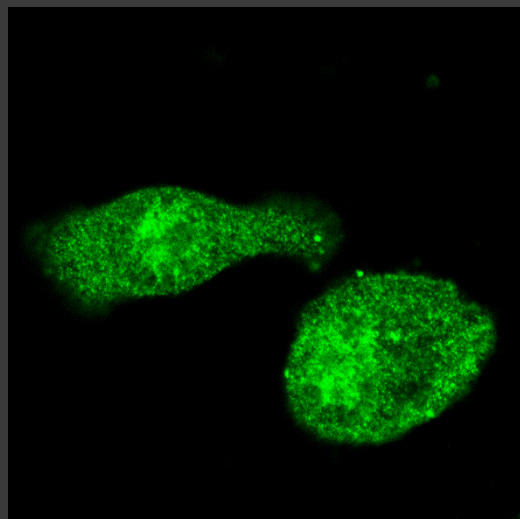
As

Process → Image

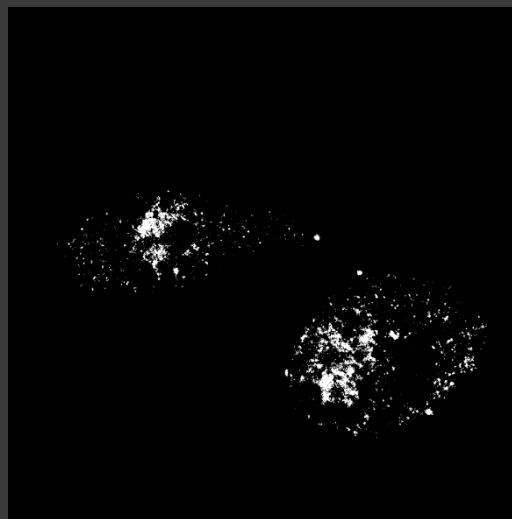
Calculator

# OBRAZOVÁ ARITMETIKA – PŘÍKLADY

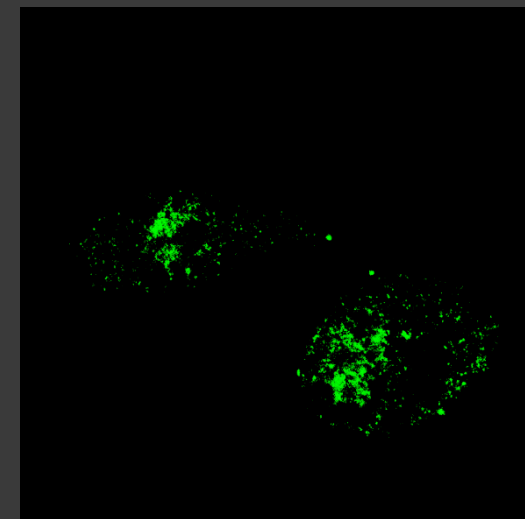
Segmentace



AND



=

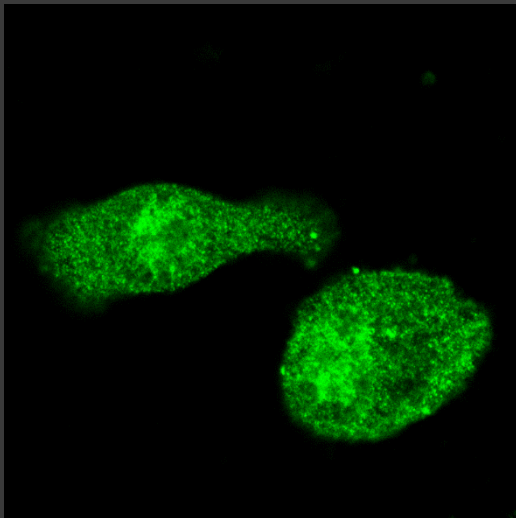


Maska

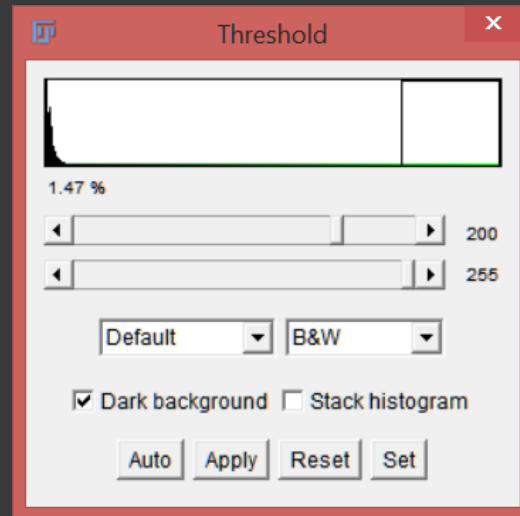
ImageJ:  
Image → Adjust →  
Threshold

# SEGMENTACE – MANUÁLNÍ PRAHOVÁNÍ TRESHOLDING

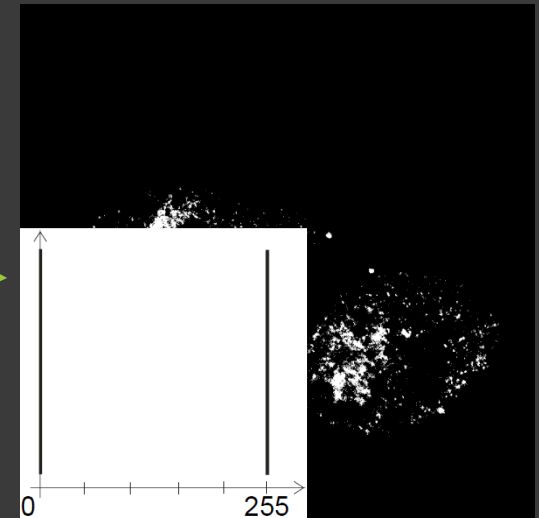
Originální obraz



Prahování



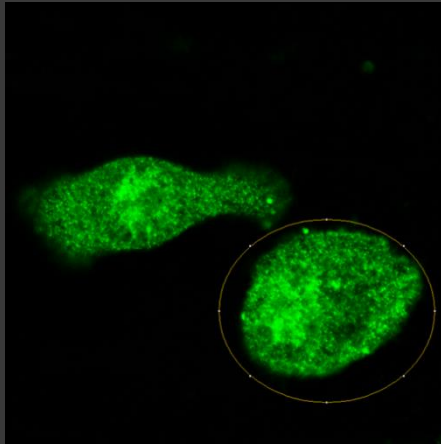
Maska



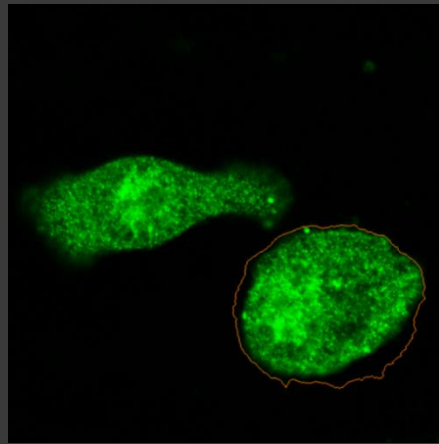
ImageJ:  
Plugins →  
Segmentation →  
Level Sets

# SEGMENTACE – POLOAUTOMATICKÁ

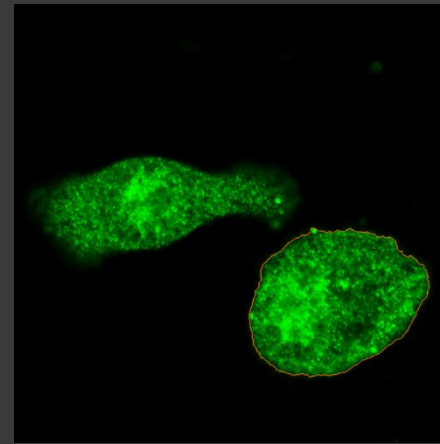
Start - ROI



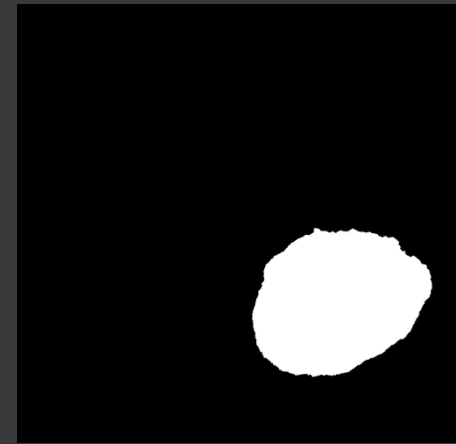
Level sets



Konec

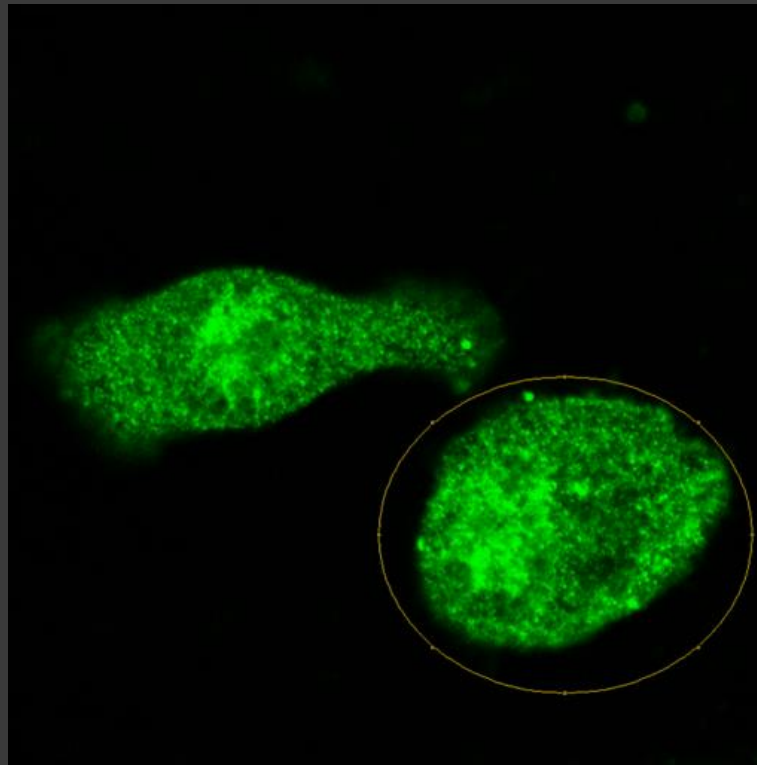


Maska



ImageJ:  
Plugins →  
Segmentation →  
Level Sets

# SEGMENTACE — POLOAUTOMATICKÁ





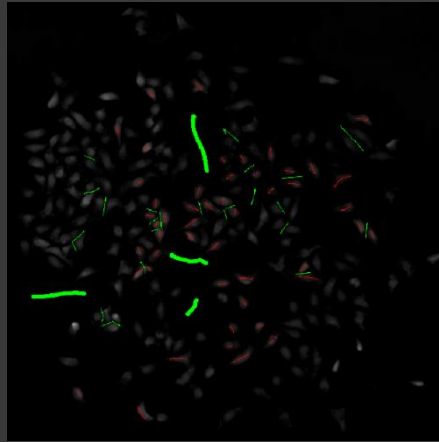
Ilustik:  
Pixel Classification  
→ Input Data →  
Feature Selection →  
Training →  
Prediction Export

# SEGMENTACE – STROJOVÉ UČENÍ

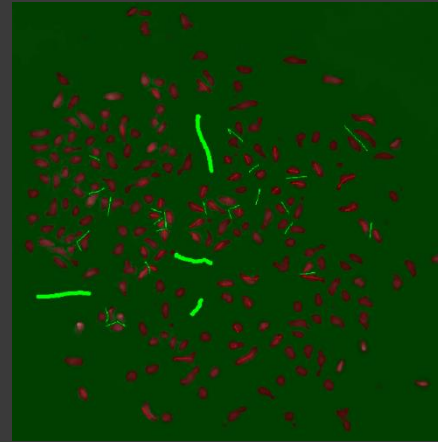
Originální obraz



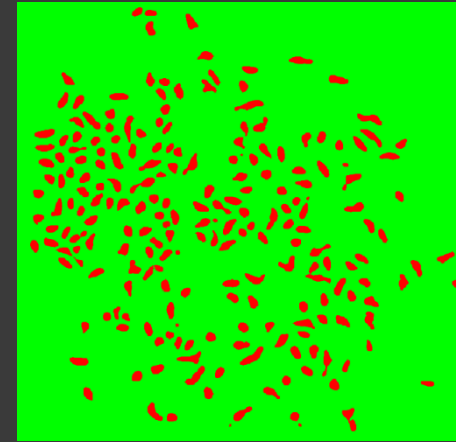
Učení malováním



Kontrola a oprava



Maska



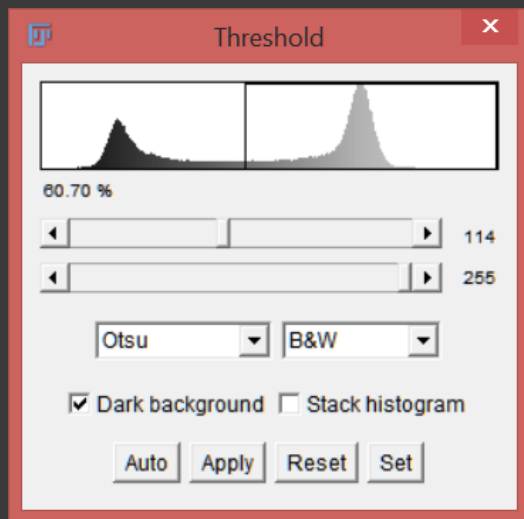
ImageJ:  
Image → Adjust →  
Threshold → Otsu

# SEGMENTACE – AUTOMATIZOVANÁ

Originální obraz

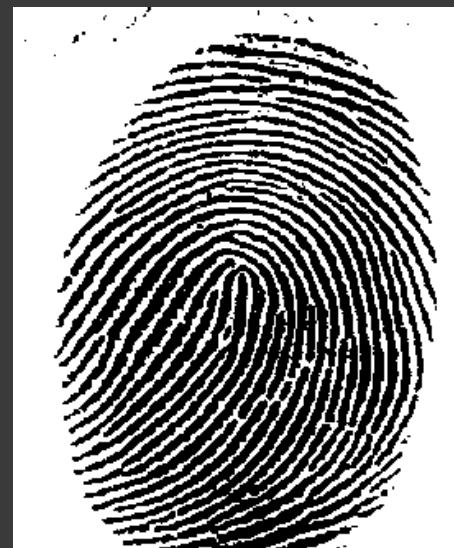


Otsu - Klastrování



Intra-class variance minimal

Segmentovaný obraz



ImageJ:

Process → Binary →

Make Binary

Process → Binary →

Dilate/Erode/Open

/Close

# ZMĚNA MORFOLOGIE MASKY

Roztažení



Otevření



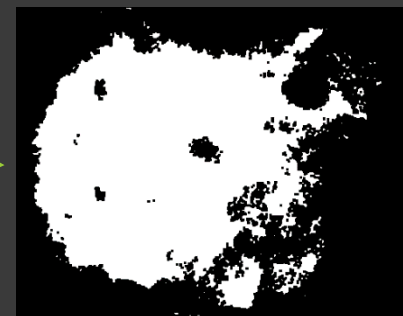
+ Structuring element



Eroze



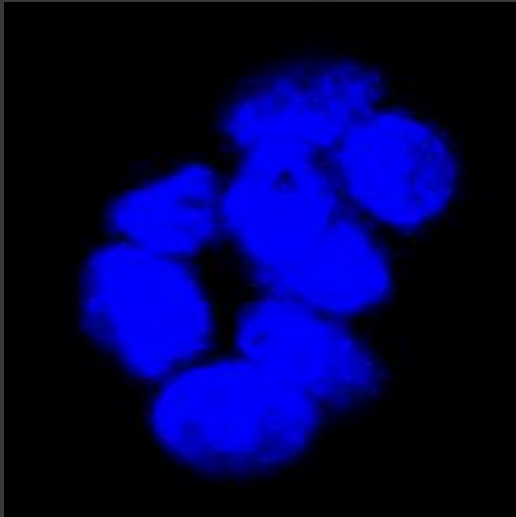
Zavření



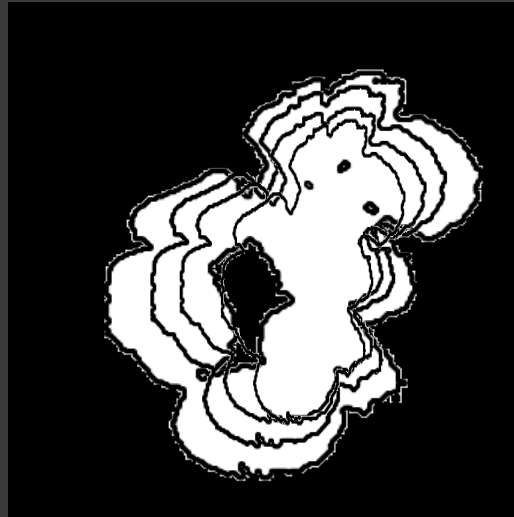
# ZMĚNA MORFOLOGIE MASKY

ImageJ:  
Process → Binary →  
Make Binary  
Process → Binary →  
Watershed

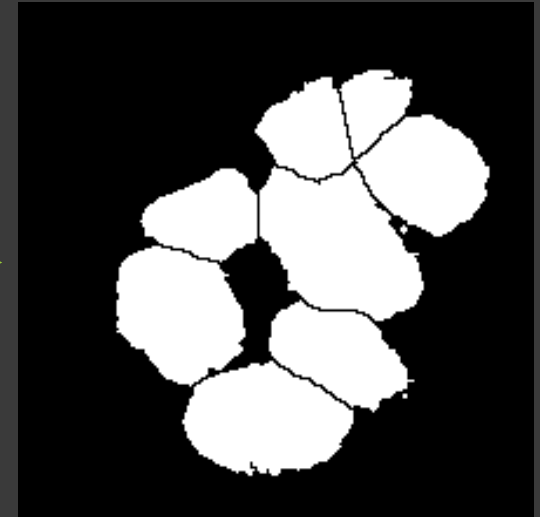
Originální obrazy



Prahování



Watershed

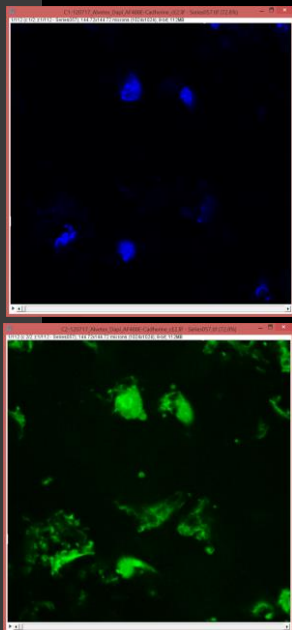


Voda stéká do údolí

ImageJ:  
Image → Adjust →  
Threshold → Otsu  
Plugins → 3D  
Viewer

# SEGMENTACE – 3D MODELOVÁNÍ

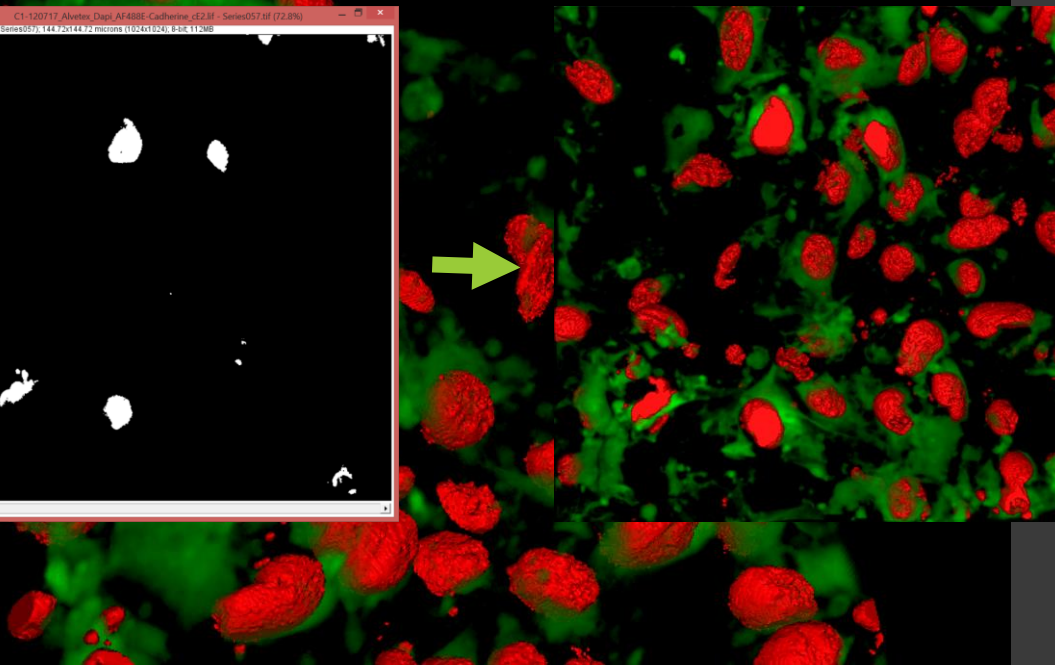
Originální obraz



Oprahovaná jádra



3D projekce



# ANALÝZA - SOFTWARE

CellProfiler

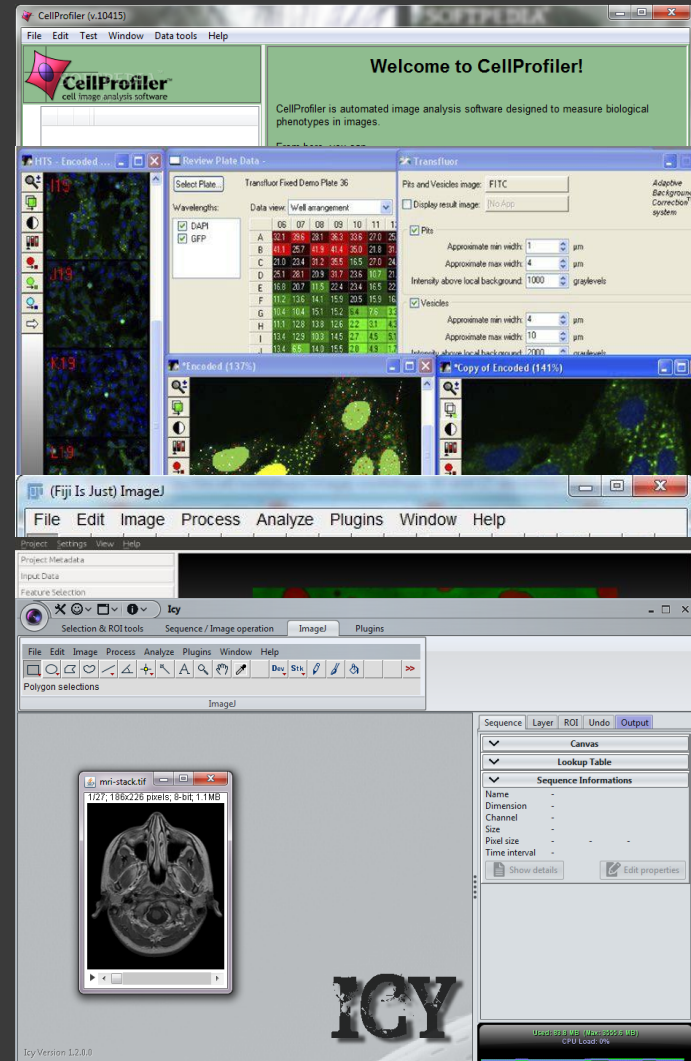
MD Metaxpress

LAS X

FIJI / ImageJ

Ilastik

Icy



ImageJ:  
Image → Properties  
Analyze → Analyze  
Particles

# OBRAZOVÁ ANALÝZA - STEREOLOGIE

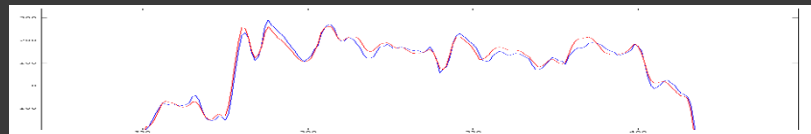
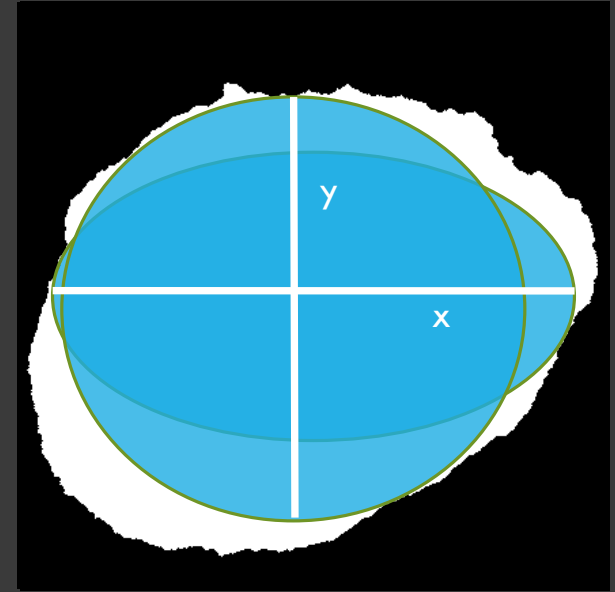
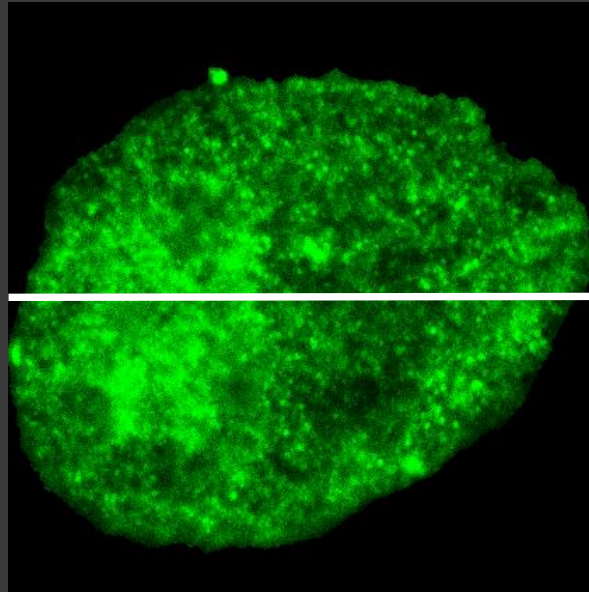
Velikost pixelu

Plocha

Intenzita (bitová hl.)

Textura

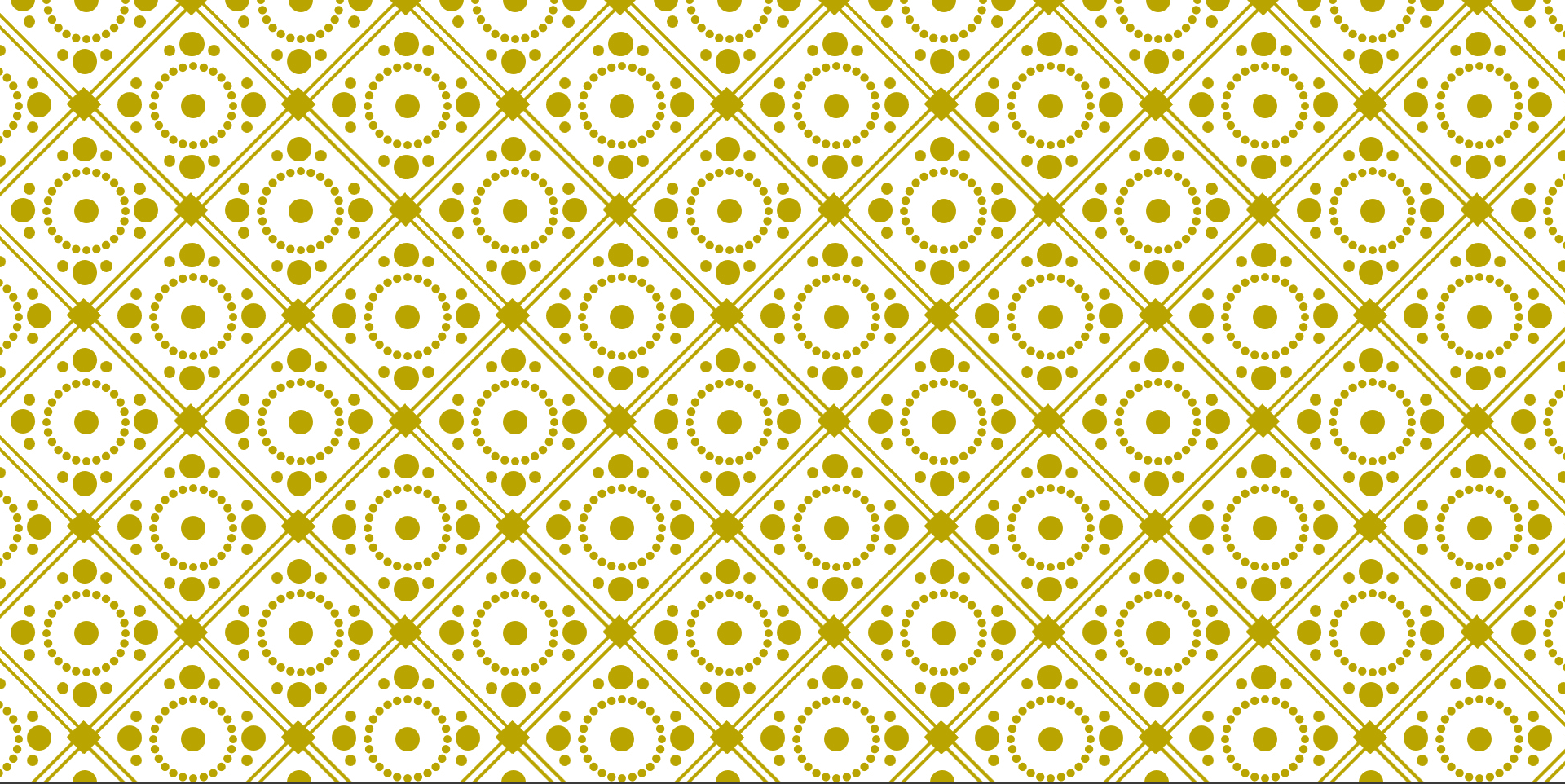
Morfologie



# PROBLÉMY

- Doba snímání
- Titrace, výběr protilátky, fluorochrom, fixace





# APLIKACE

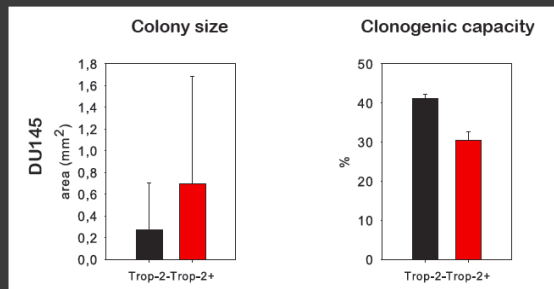
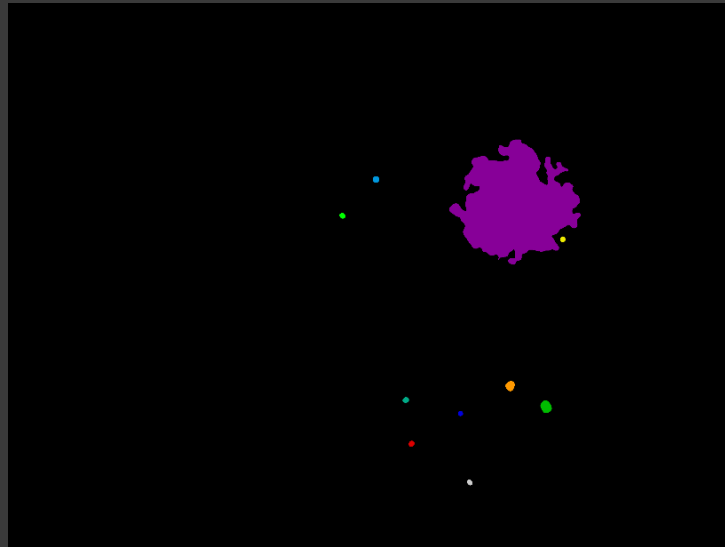
Příklady analýz

PRAKTICKÁ UKÁZKA

ANALÝZA SFEROIDŮ

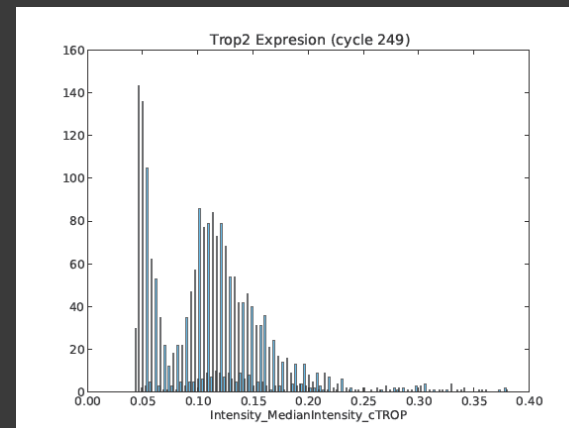
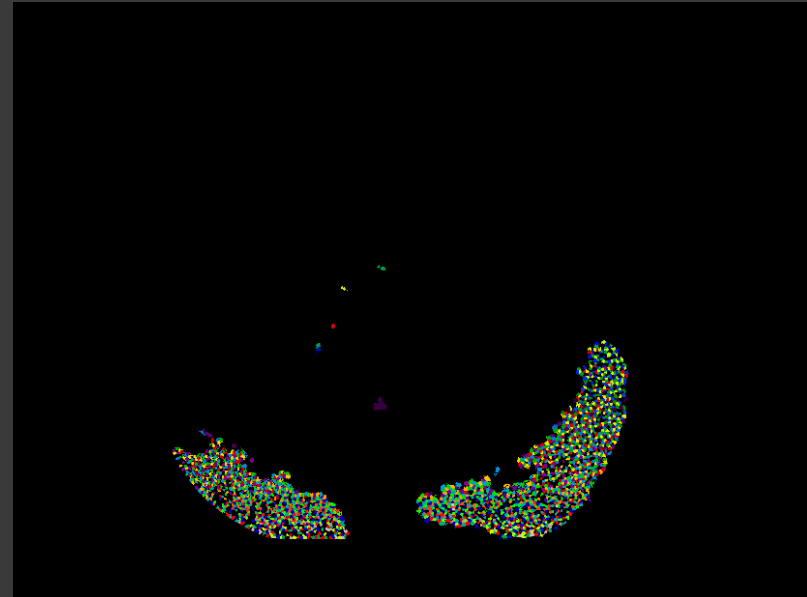
# POČÍTÁNÍ BUNĚČNÝCH KOLONIÍ

- Highthroughput
- Průchozí světlo
- Velikost kolonie
- Klonogenní kapacita
- Testování léčiv

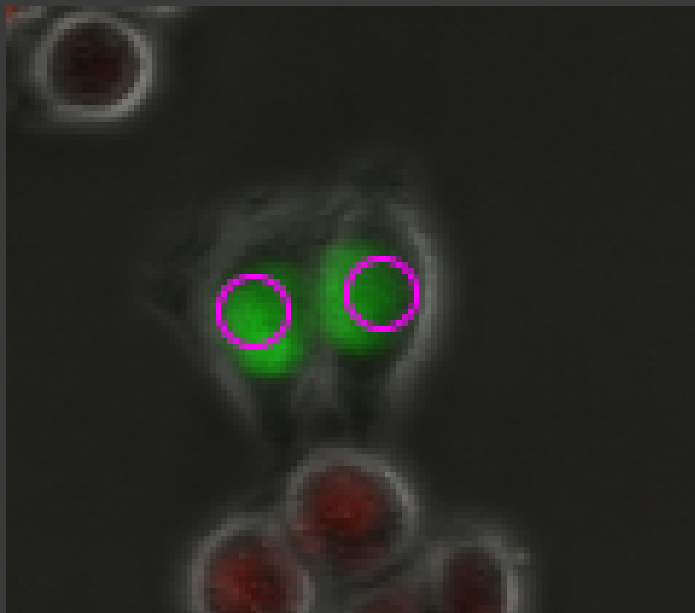
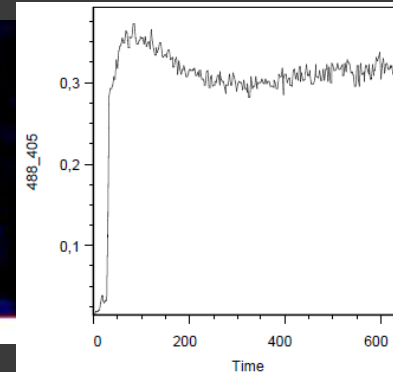
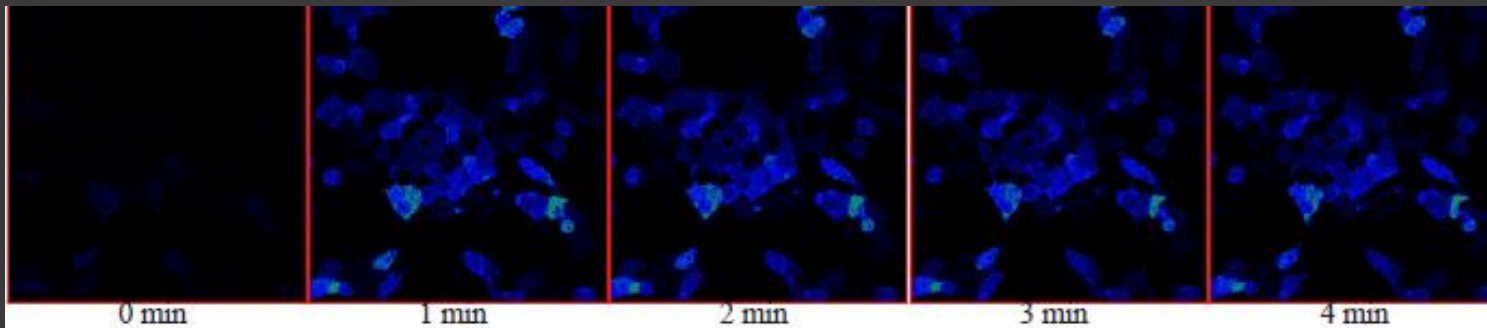


# KVANTIFIKACE POVRCHOVÝCH MARKERŮ

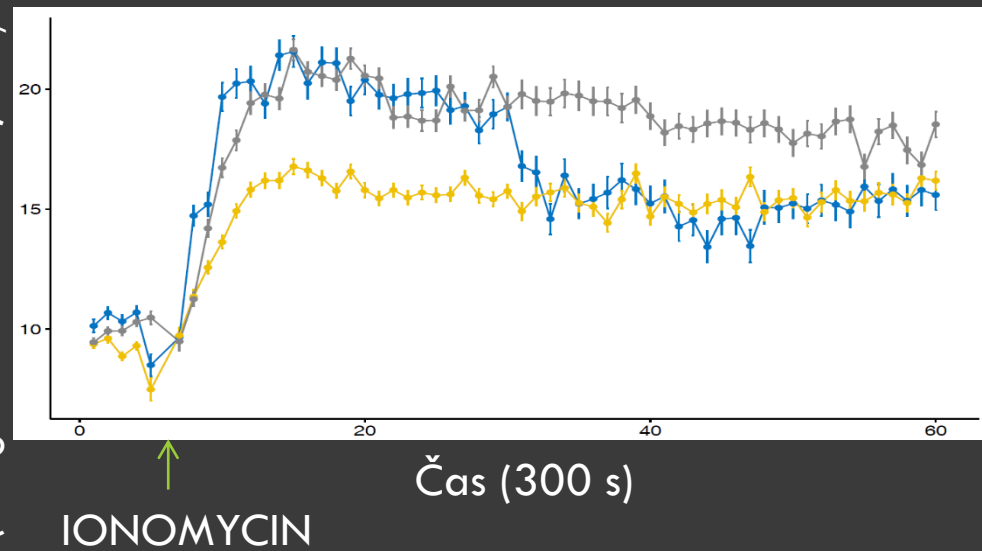
- Hightroughput
- Jednotlivé buňky
- Průchozí světlo + Fluorescence
- Plasticita TROP-2
- Automatizovaná procedura
- Zpracování dat - histogram



# ANALÝZA ŽIVÝCH BUNĚK

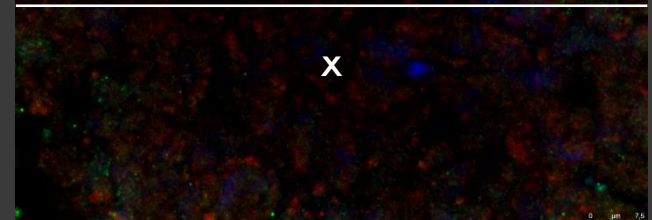
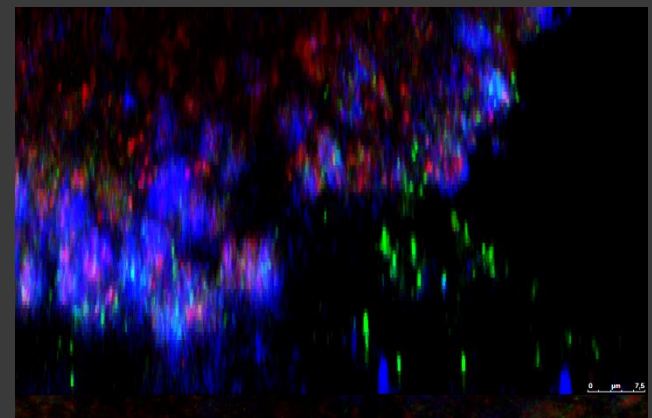
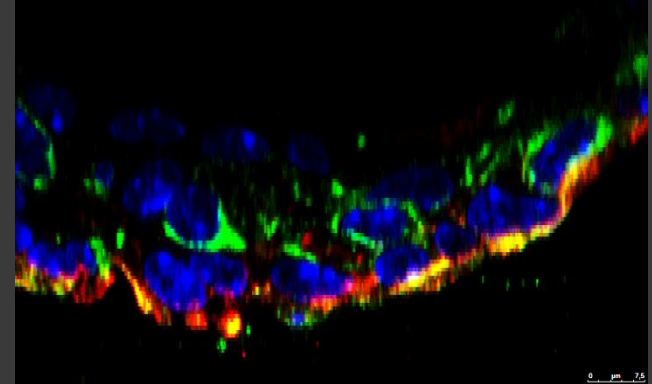
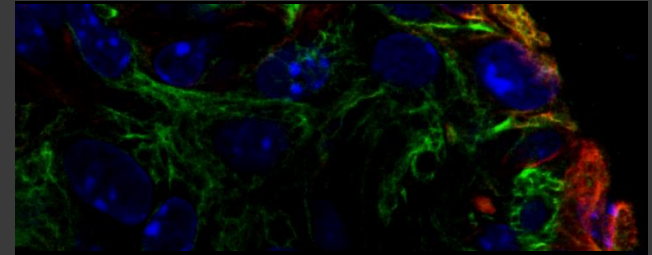
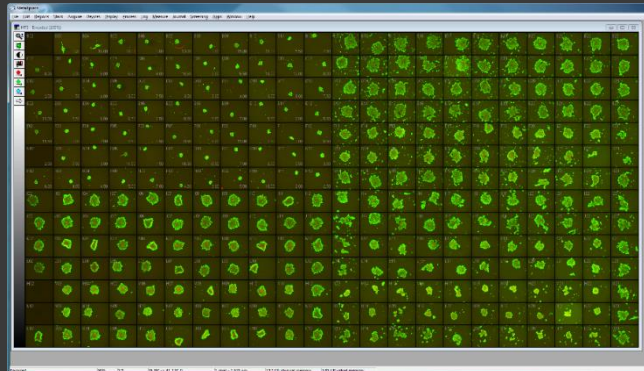
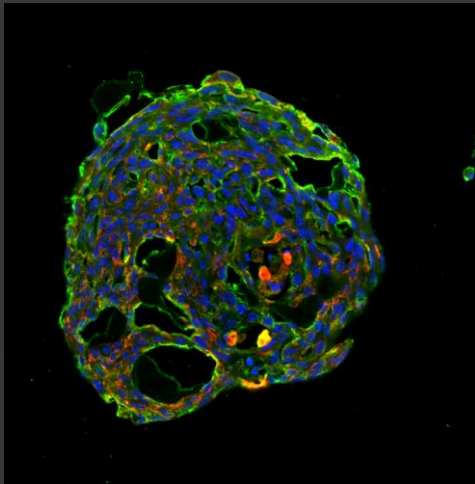
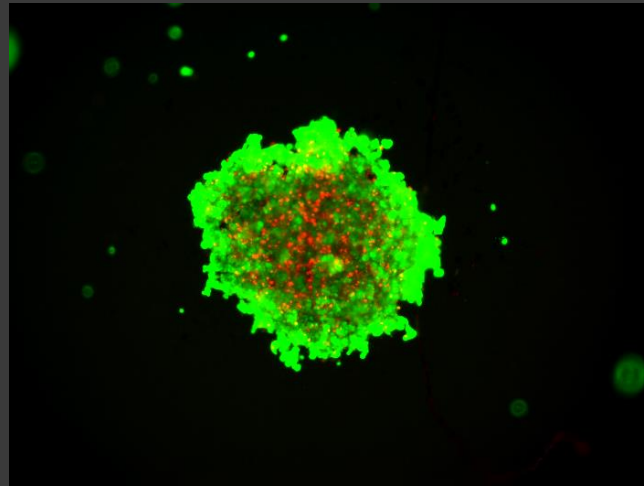
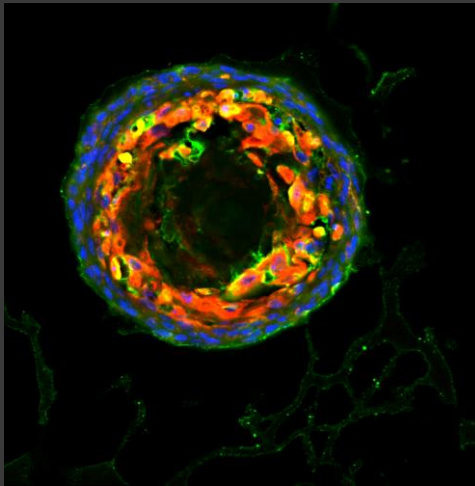


Fluo-4  
(Integrovaná intenzita, a.u./buňka)



# 3D ANALÝZY

- Kvantifikace objemu
- 3D projekce
- 3D kultivace



Z

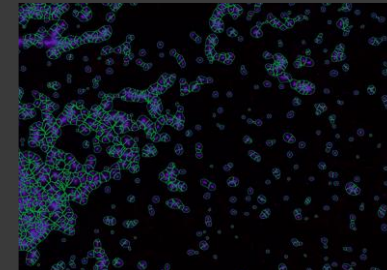
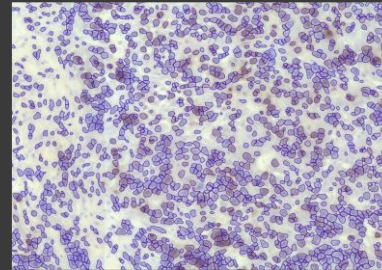
X

# DETEKCE SFEROIDŮ A INVAZIVITY

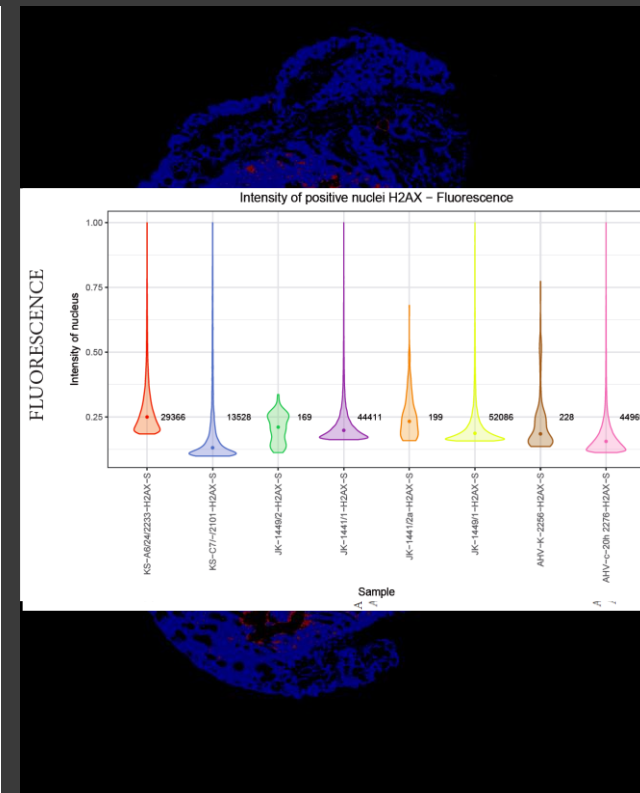
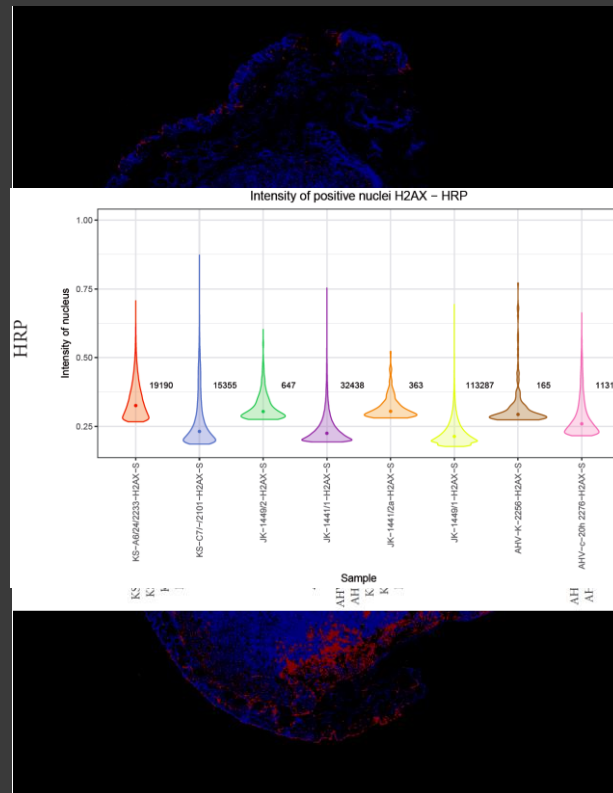
- Hightroughput
- Průchozí světlo
- Detekce sferoidů
- Detekce invadujících buněk
- Plocha



# ANALÝZA TKÁNÍ



- Hightroughput
- Barevný obraz
- Fluorescence
- Detekce jader
- Kvantifikace intenzity
- Zpracování dat - R

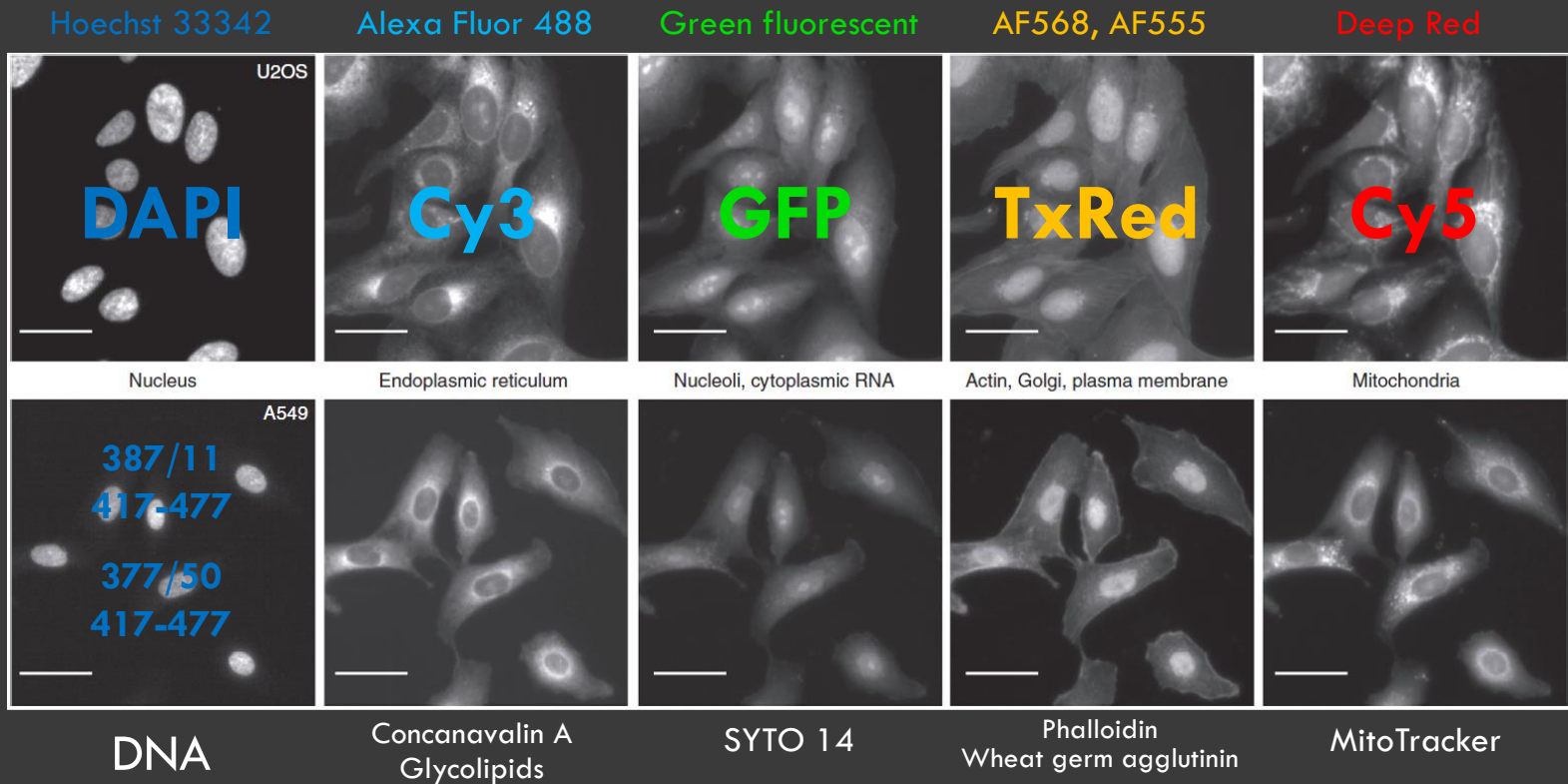




# MORFOLOGIE — CELL PAINTING ANALÝZA

- **Nature protokol**
  - BRAY, Mark-Anthony, et al. Cell Painting, a high-content image-based assay for morphological profiling using multiplexed fluorescent dyes. Nature protocols, 2016, 11.9: 1757.
- **Vysokokapacitní analýza obrazu pro vytvoření morfologického profilu buněk**
- **6 fluorochromů/protilátek**
- **5 kanálů**
- **8 buněčných kompartment/organel**
- **~1,500 morfologických parametrů**
- **Kultivace & Snímání obrazu = 2 týdny + počítání vlastností & analýza dat = 2 týdny**

# CELL PAINTING — PROTILÁTKY, FLUOROCHROMY



# CELL PAINTING - CÍLE

- **Kvantifikace morfologie a rozdílů mezi buněčnými liniemi/klony**
- **Morfologický „otisk“ (profil) buněk**
- **Výběr parametrů s největší odlišností pro jednotlivé linie**

# CELL PAINTING - POSTUP



# CELL PAINTING - VÝSTUPY

## Obrázky

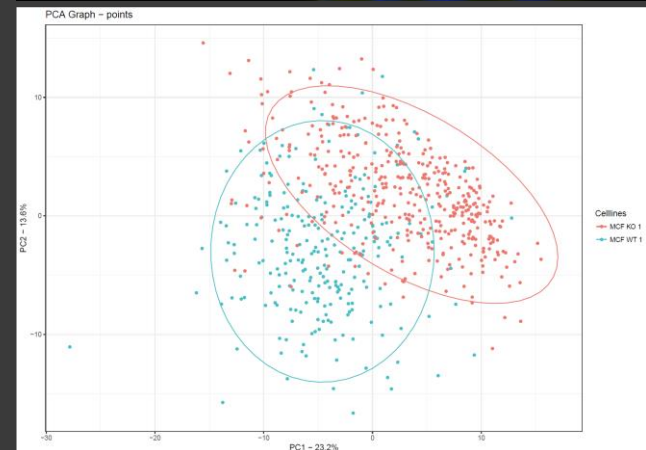
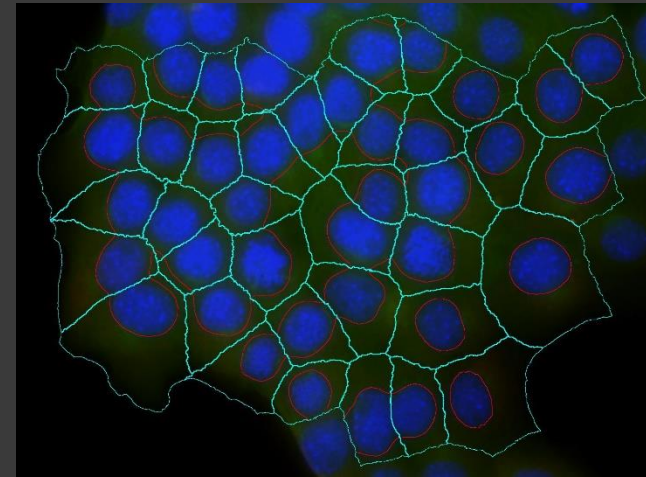
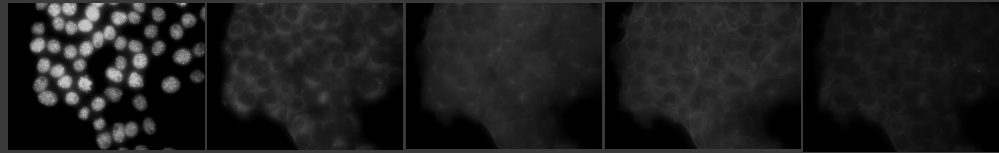
- 16bit a 12bit šedotónové
- Kontrolní obrazy segmentace
- 240GB dat – 1 opakování

## Grafy

- Heatmapa
- PCA graf
- t-SNE graf

## 3 Textové soubory & Excel tabulka

- Jádro, Cytoplazma, Buňka
- přibližně 600 parametrů z každého objektu
- 10 nejvýznamějších parametrů



# CELL PAINTING - OBRÁZKY

DAPI

Cy3

GFP

TxRed

Cy5

Hoechst 33342

Alexa Fluor 488

Green fluorescent

AF568, AF555

Deep Red

Merge

316

317

318 ctrl

DNA

Concanavalin A  
Glycolipids

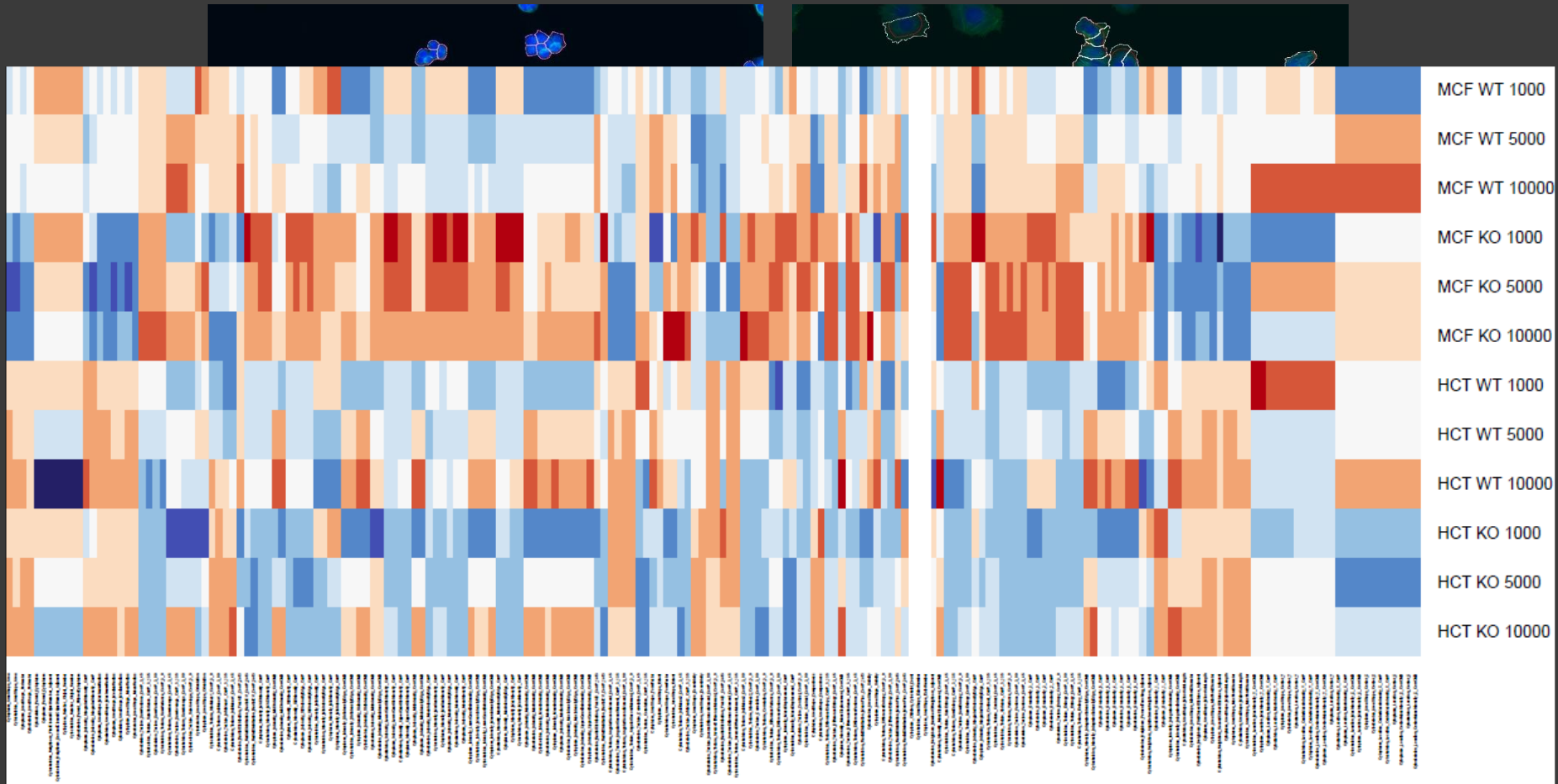
SYTO 14

Phalloidin  
Wheat germ agglutinin

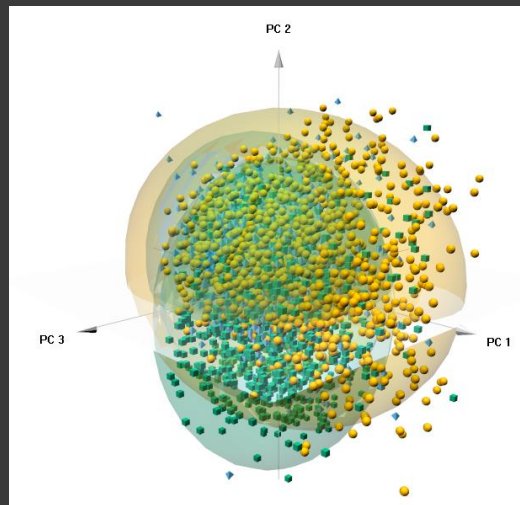
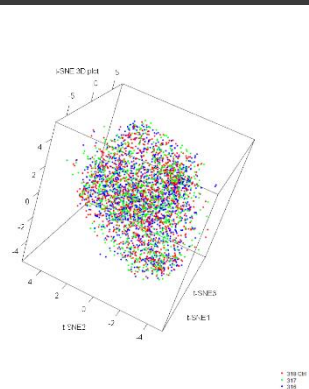
MitoTracker

Hoechst, Concanavalin  
A, MitoTracker

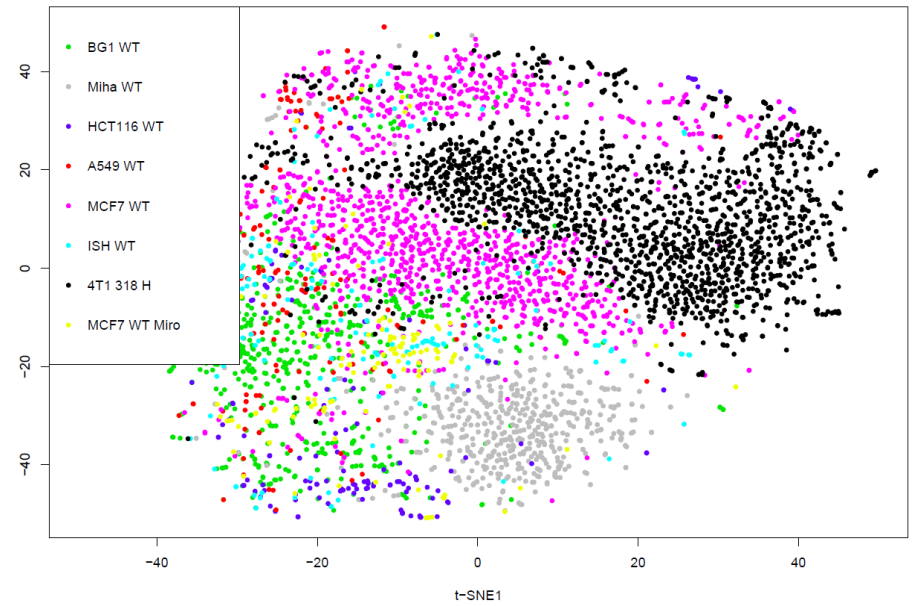
# CELL PAINTING - HEATMAP



# CELL PAINTING — PCA, T-SNE



t-SNE plot



PCA Graph - points





IMAGEJ

FIJI

CELLPROFILER

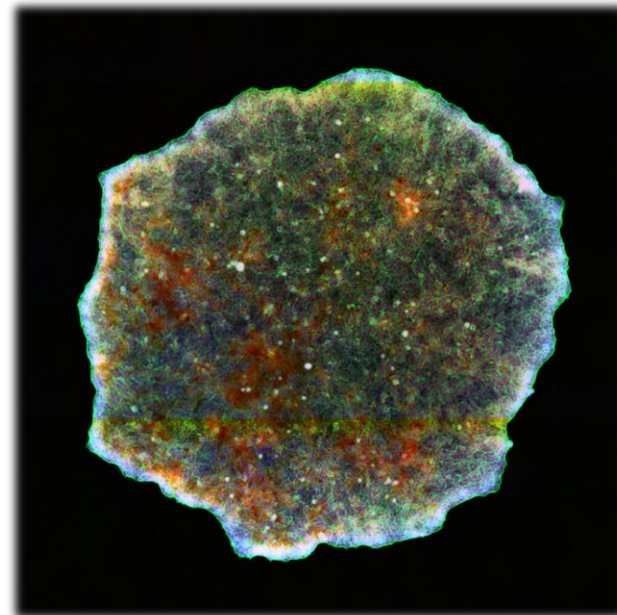
ILASTIK

<http://imagej.nih.gov/ij/>

<http://fiji.sc/>

<http://www.cellprofiler.org/>

<http://ilastik.org/>



Co-funded by the  
7<sup>th</sup> Framework Programme  
of the European Union

✓  
DĚKUJI ZA POZORNOST

27.11.2018, 4.12.2018

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