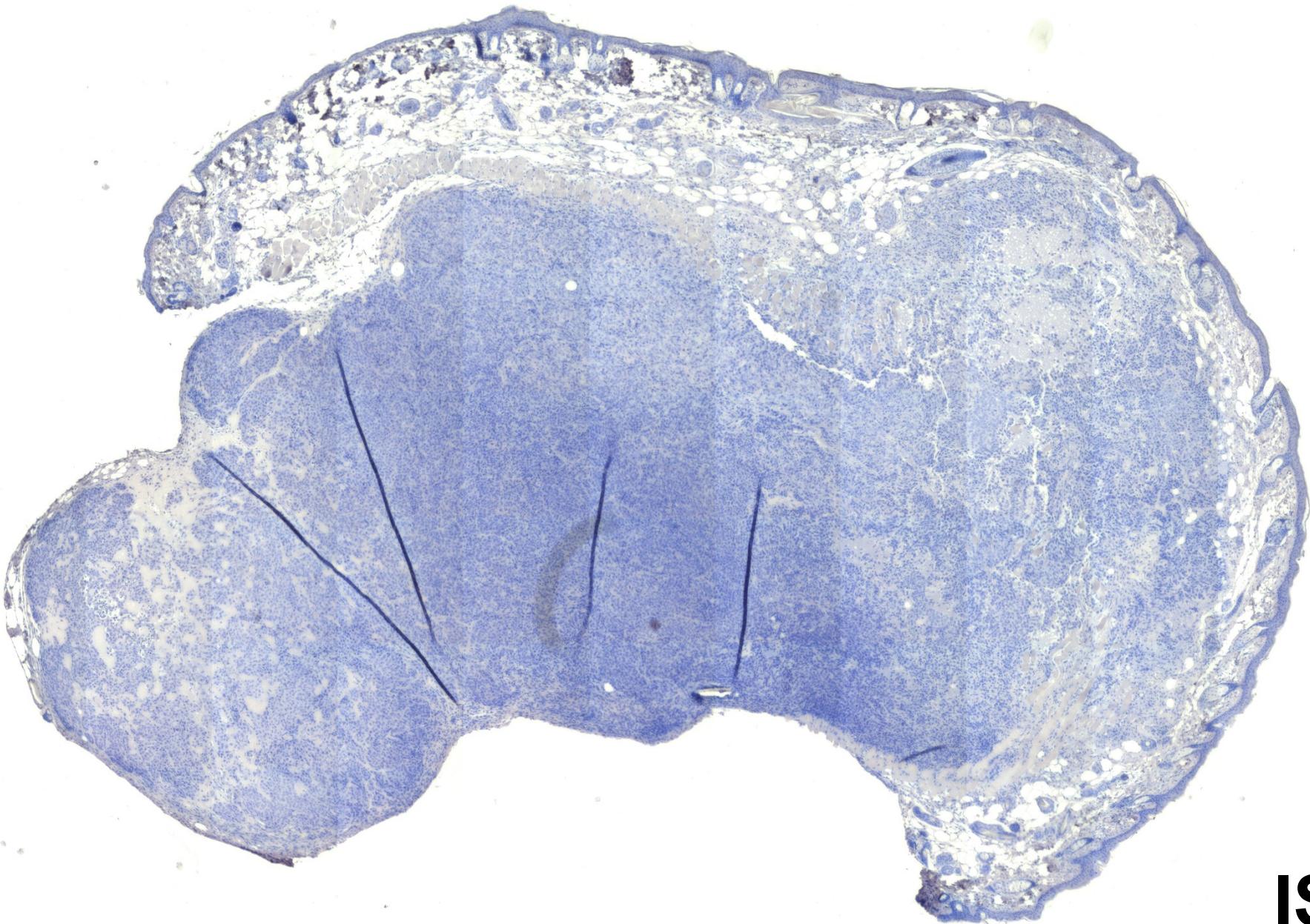




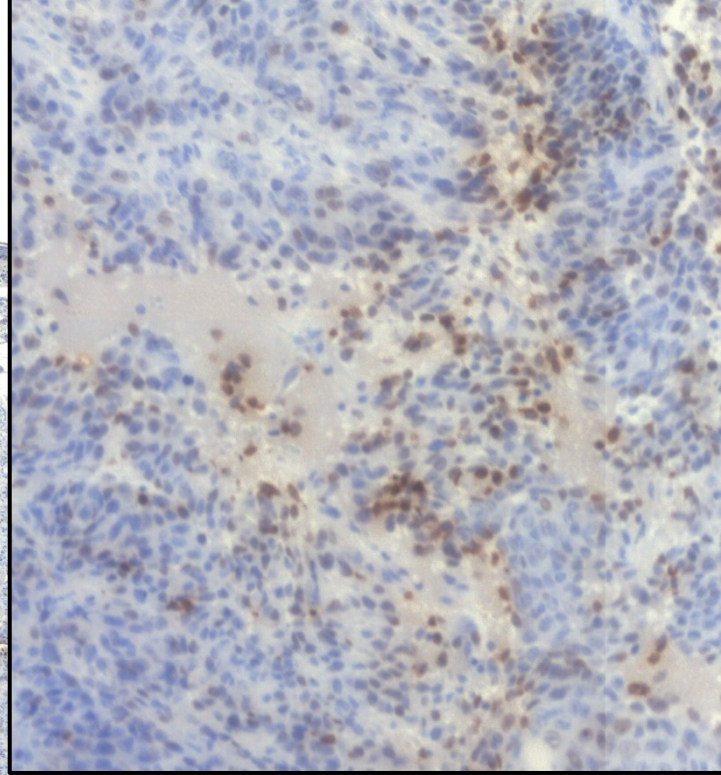
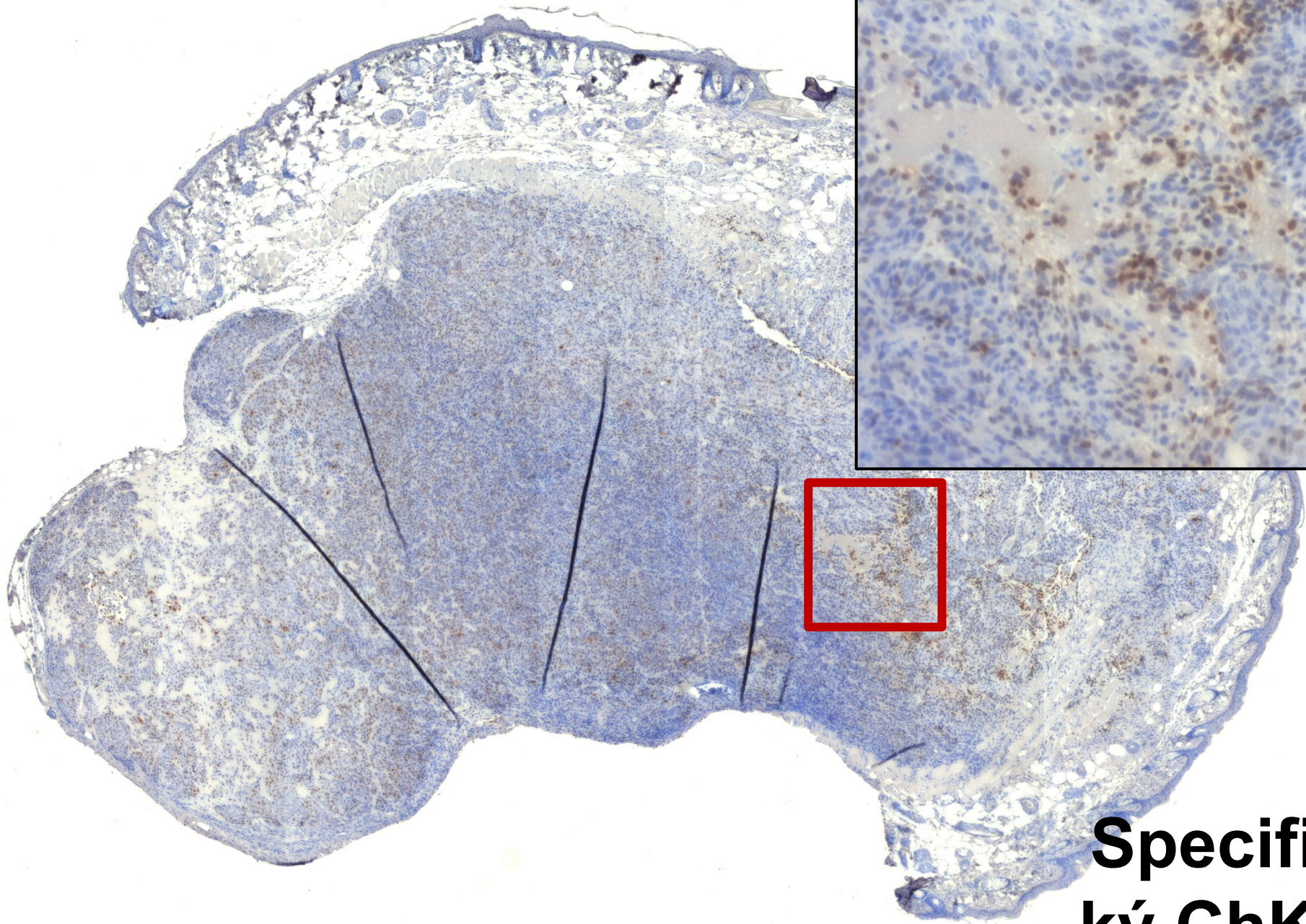
ZÁKLADNÍ PRINCIPY ANALÝZY OBRAZU

Příklady analýzy
obrazu

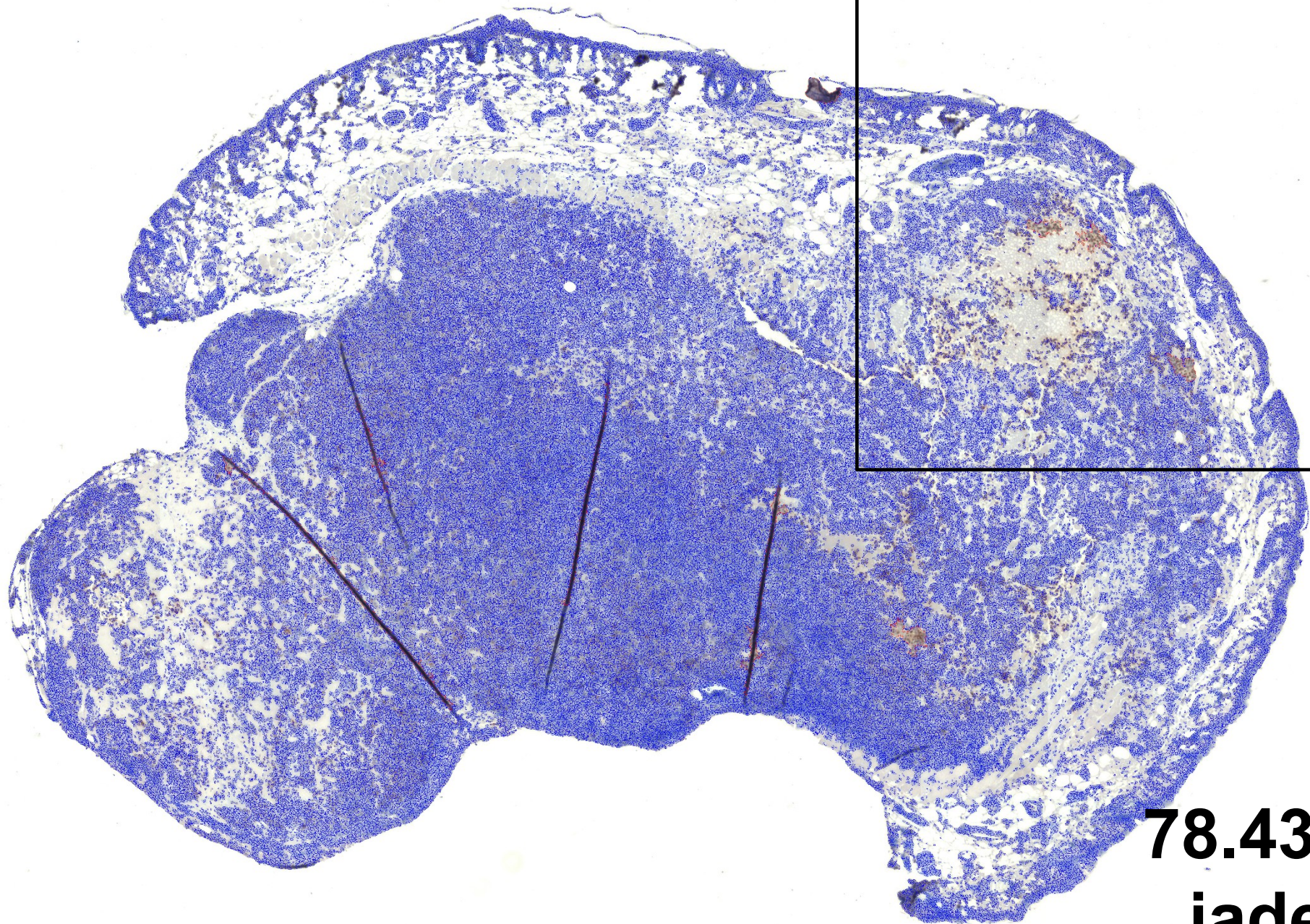
Radek Fedr



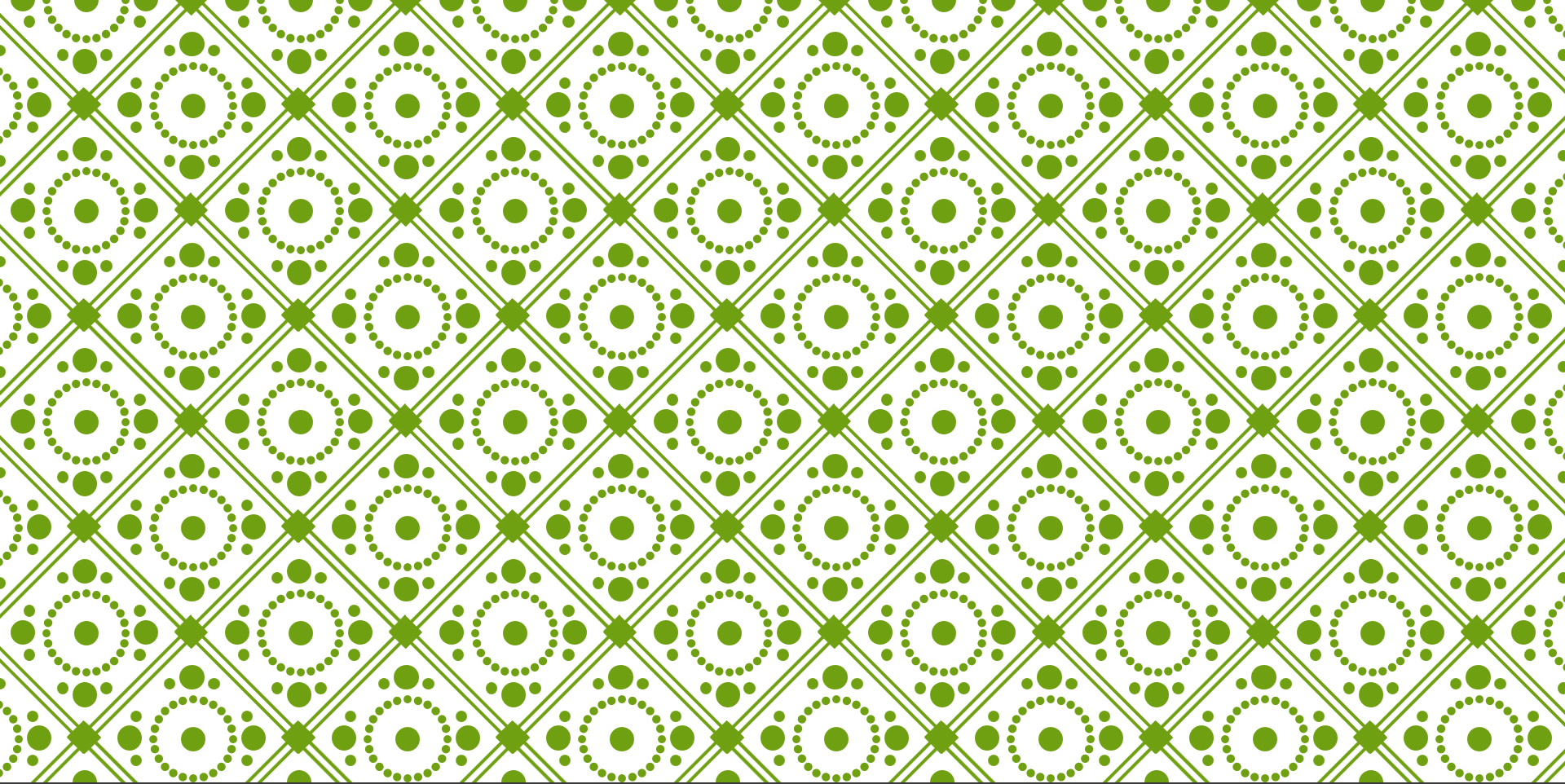
ISO



**Specific
ký ChK1**



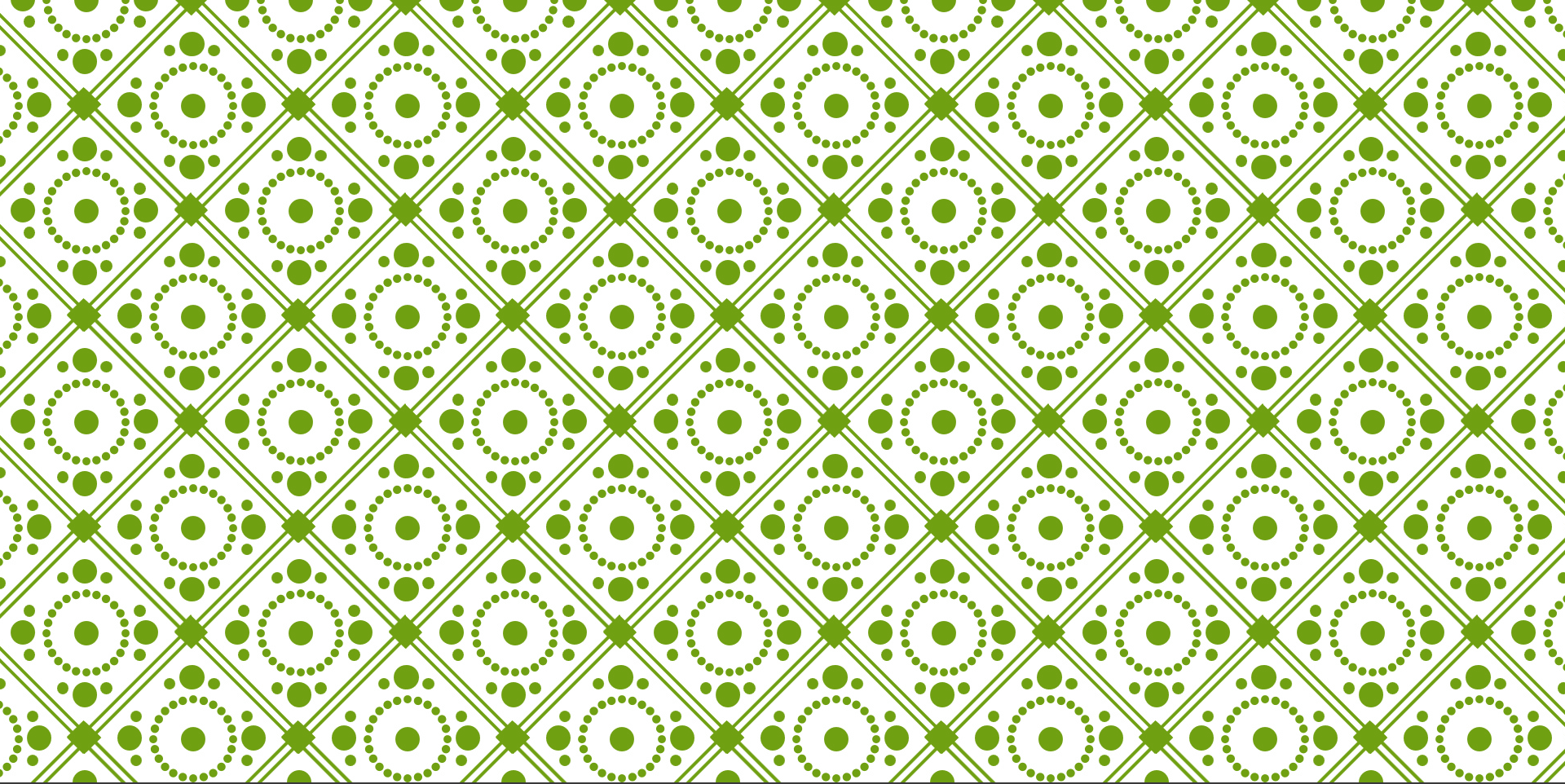
78.436
jader



ANALÝZA TKÁNĚ

- 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

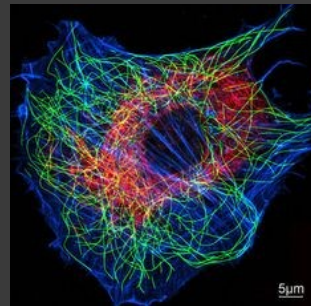




SROVNÁNÍ

Mikroskopie

Ukázka



Vzorek

2D, 3D

Rozlišení

100nm

Fluorescenční parametry

1-5

Rychlost

100 buněk/s

Data

100GB

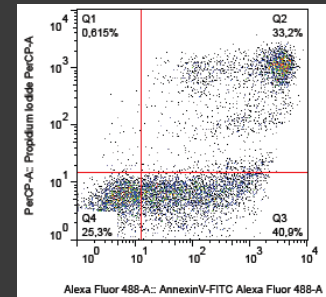
Kvantifikace

Přesná

Počet buněk

10^1 - 10^6

Průtoková cytometrie



Suspenze

1částice/1µm

10+

10000 buněk/s

100MB

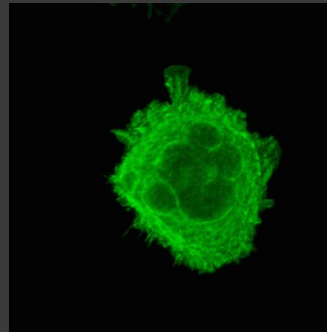
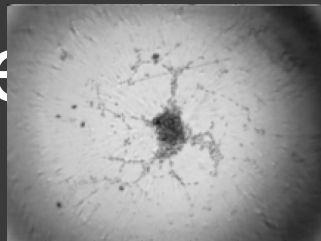
Přesná

10^4 - 10^7

PŘÍPRAVA A SNÍMÁNÍ

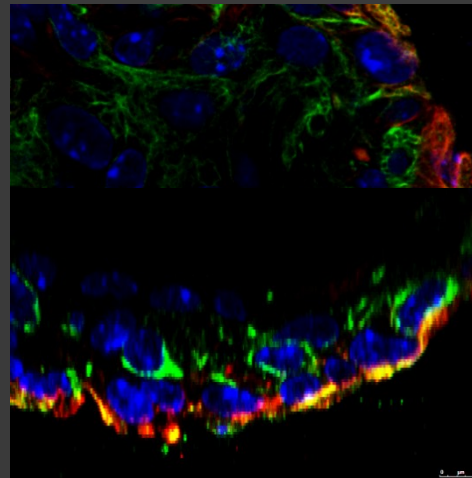
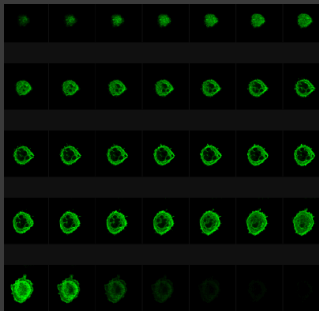
Experiment

- živé
- fixované
- 2D
- 3D



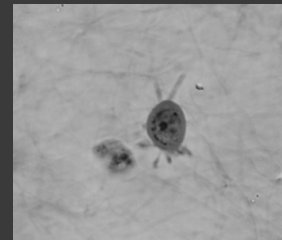
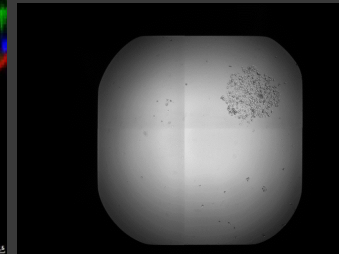
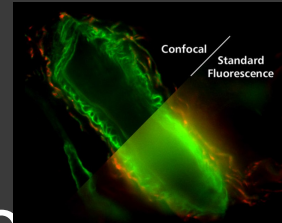
Vzorek

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

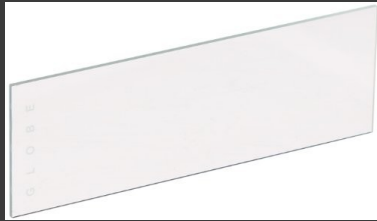


Mikroskopie

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



PŘÍPRAVA A SNÍM



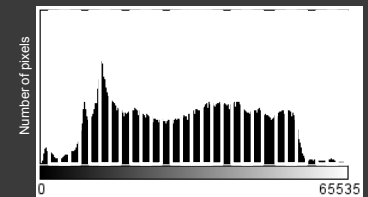
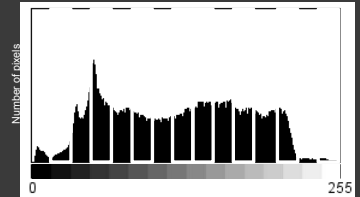
Plast

- podložní sklo
- ztenčené dno
- více-jamkové desky

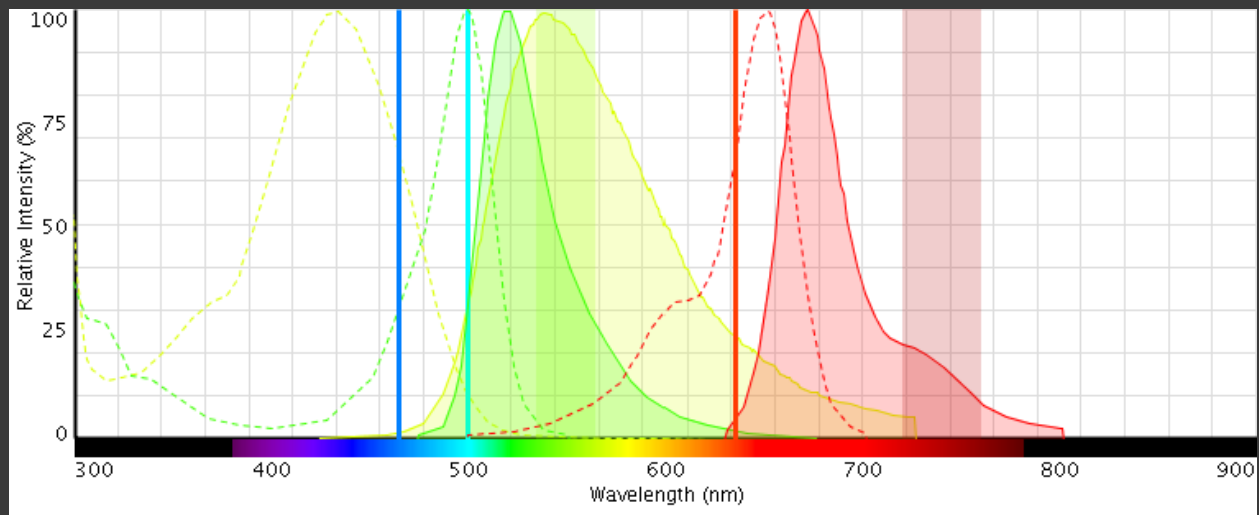
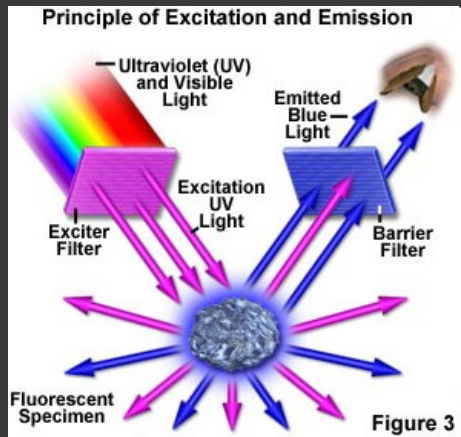
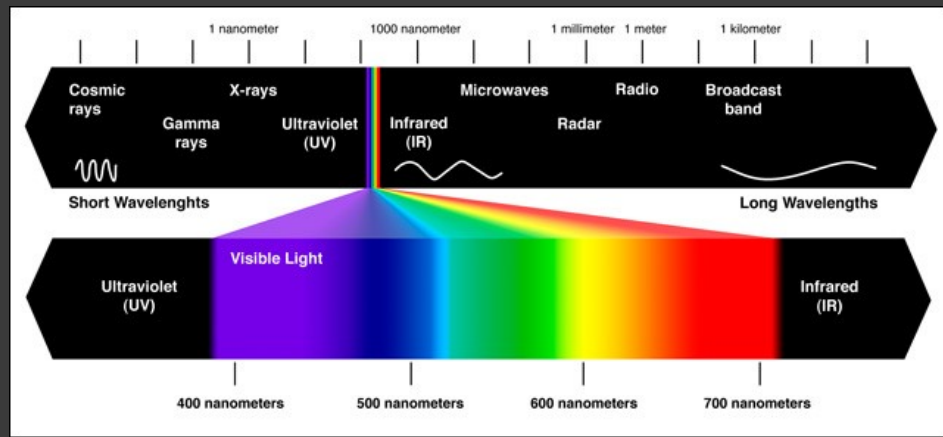
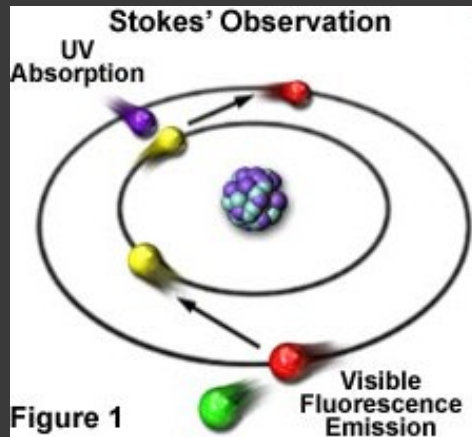


Rozlišení

- objektiv
- bitová hloubka
- počet barev

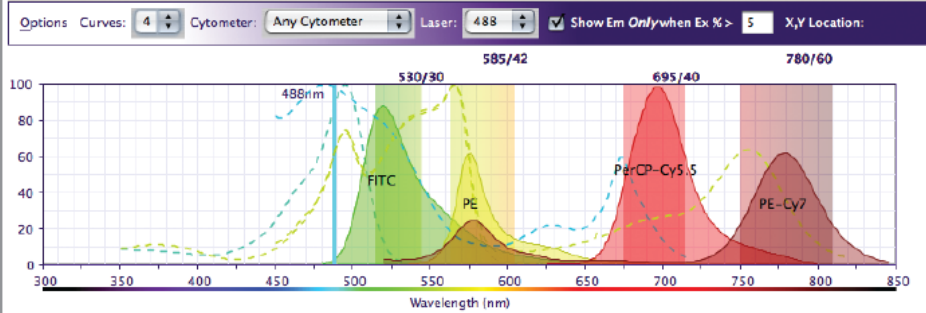


FLUORESCENCE



FLUOROCHROM

BD Fluorescence Spectrum Viewer A Multicolor Tool



- Síla fluorescence fluorochromů není stejná!

- spectraviewer

- www.fluorofinder.com



tips on selecting markers and criteria

+ Add a dump channel

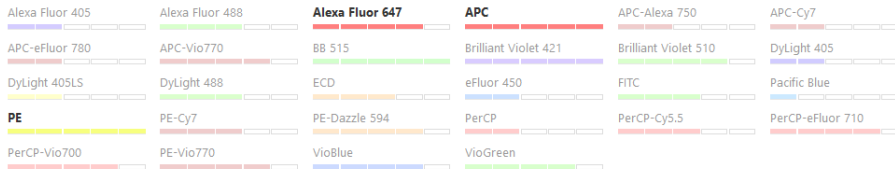
* Marker / Antigen	* Target Species <input checked="" type="checkbox"/> Batch	Host Species	Isotype	Clone	Antigen Density
--------------------	---	--------------	---------	-------	-----------------

Channels 8

CD24 Human Mouse IgG1 SN3

+ primary/secondary

Fluorochrome Availability:

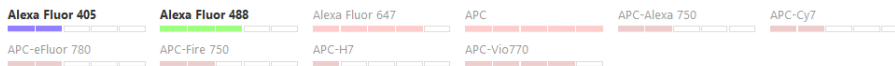


< less

CD44 Human Mouse IgG2a Any

+ primary/secondary

Fluorochrome Availability:

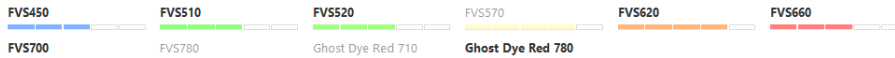


∨ more

Live/Dead All Species Any Any Any

+ primary/secondary

Fluorochrome Availability:



∨ 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



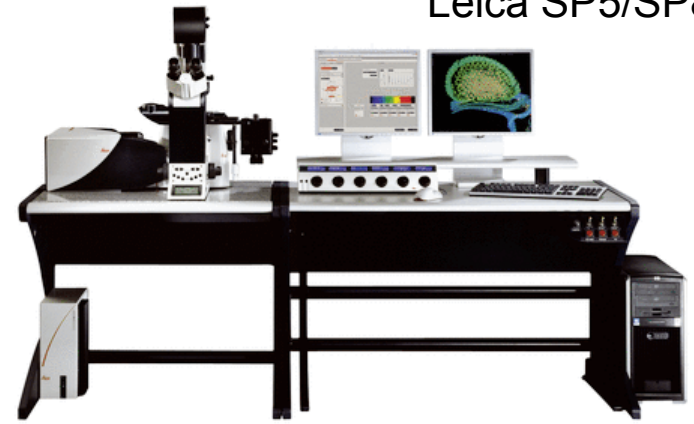
- Příprava
- Automatické
- Zpracování vzorku
- Reproštické
- Xylohydrické

“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.”

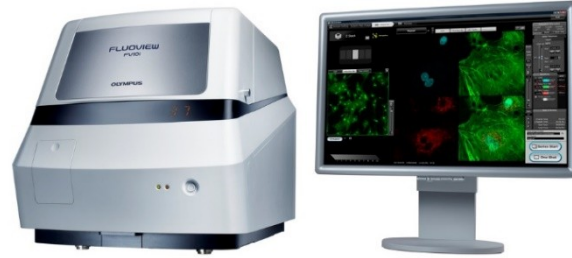
Bill Gates

MIKROSKOP

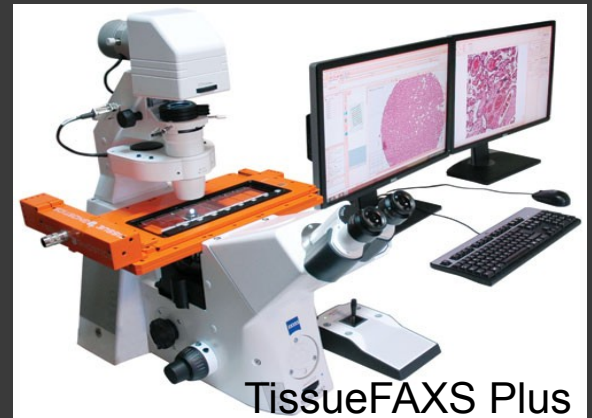
Leica SP5/SP8



Olympus/Leica



Olympus FV10i



TissueFAXS Plus

Olympus IX70

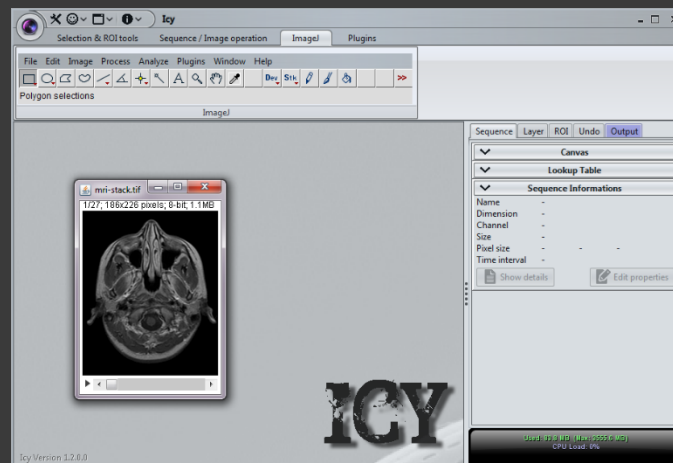
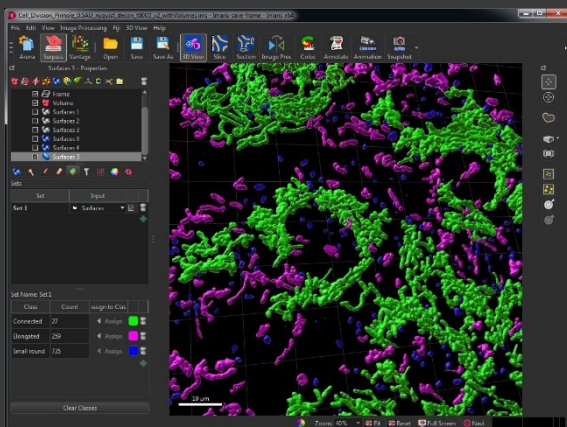
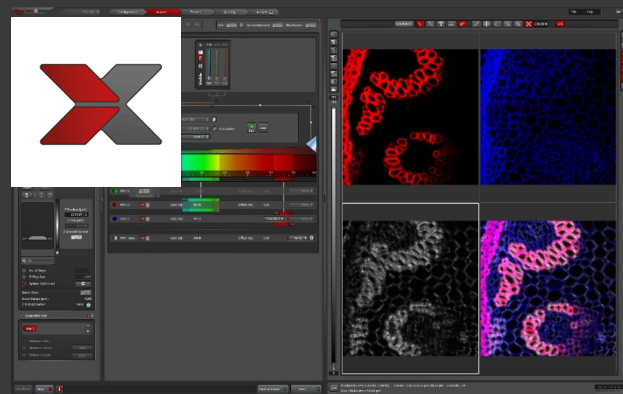
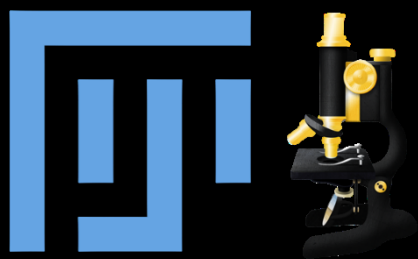


MD ImageXpress
Micro



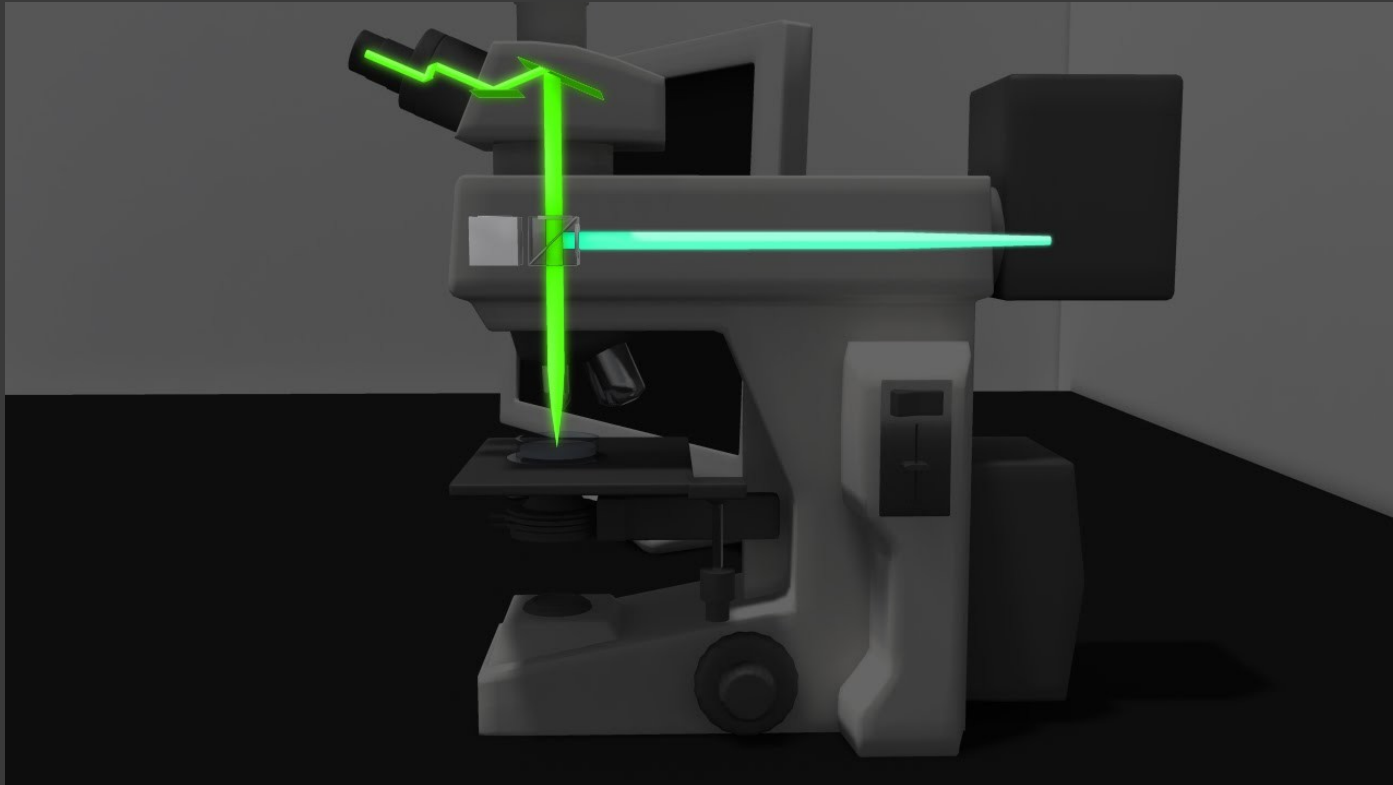
Hyperion

SOFTWARE



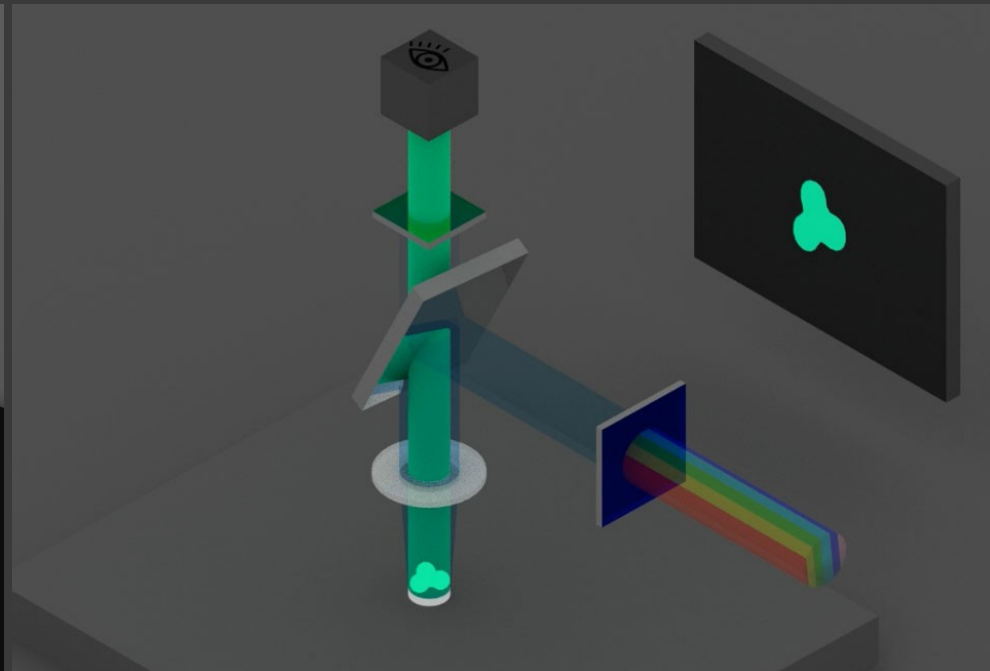
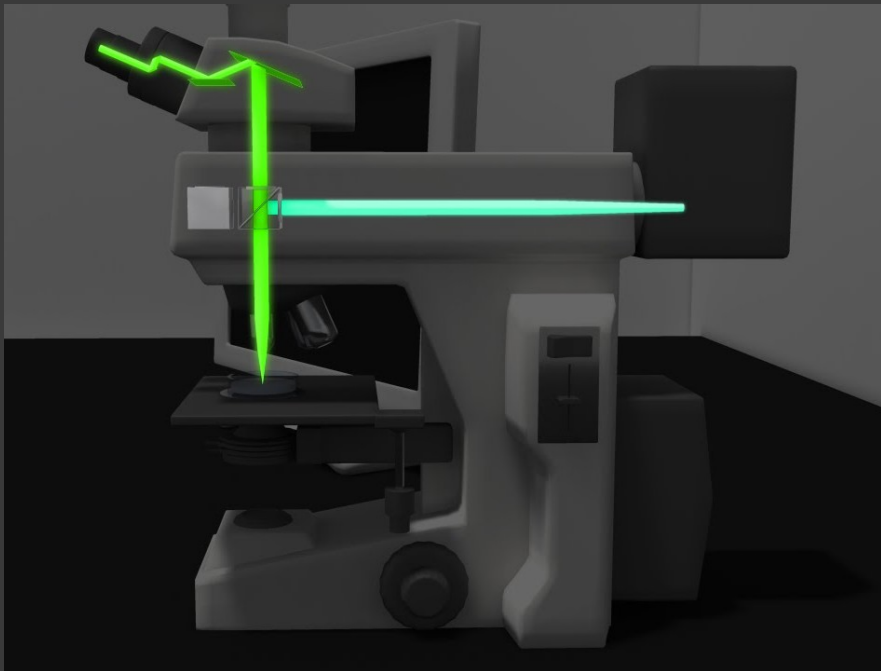
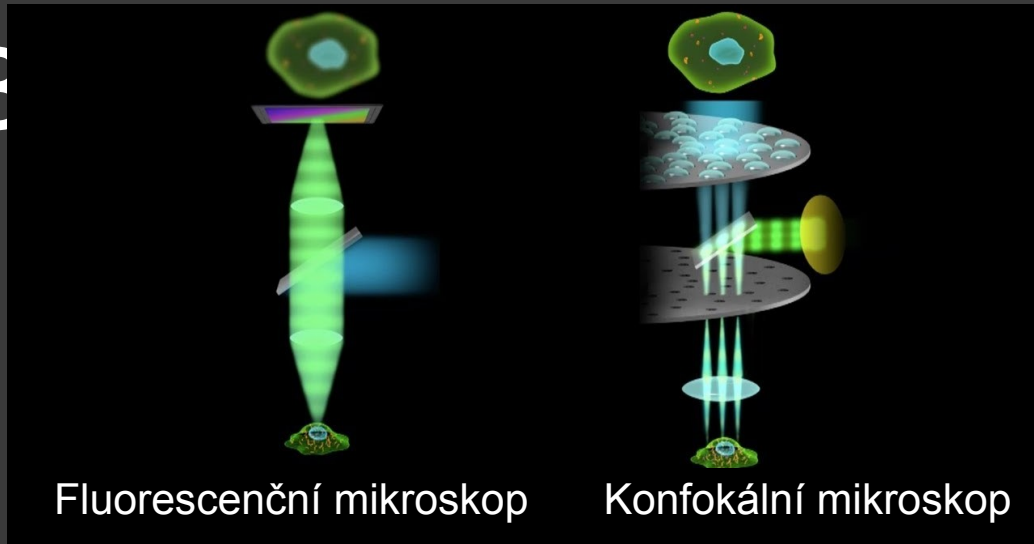
SNÍMÁNÍ OBRAZU

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

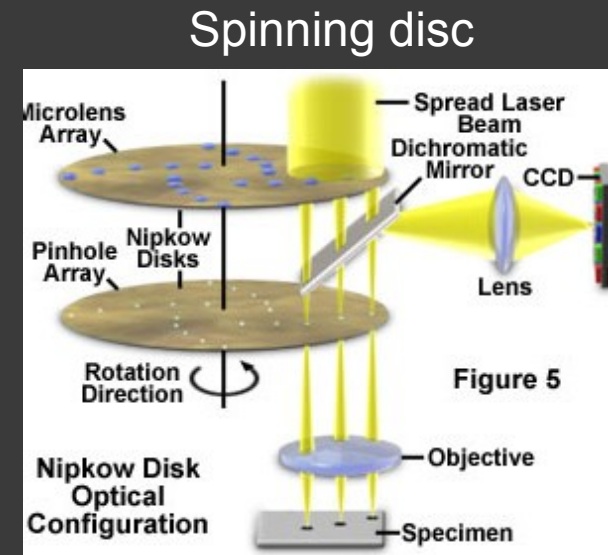
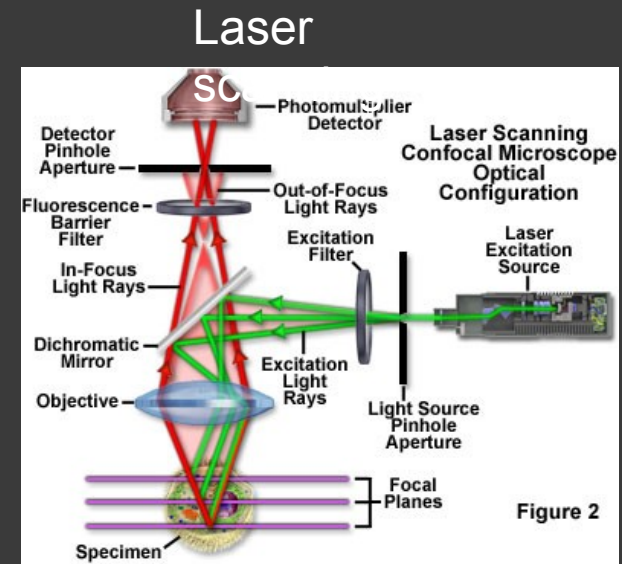
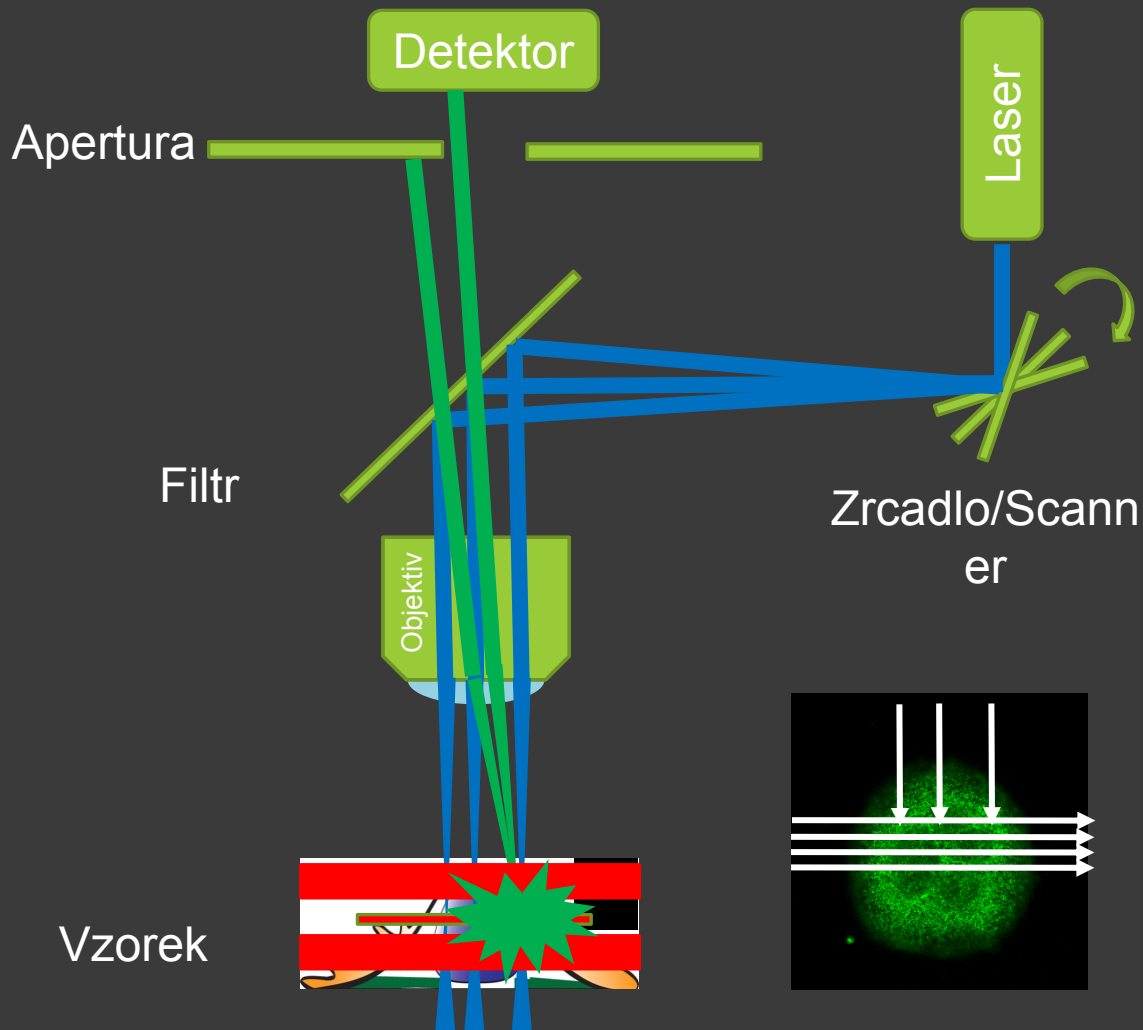


SNÍMÁNÍ OB

- Fluorescenční mikroskop
- vs.
- Konfokální mikroskop



SNÍMÁNÍ OBRAZU

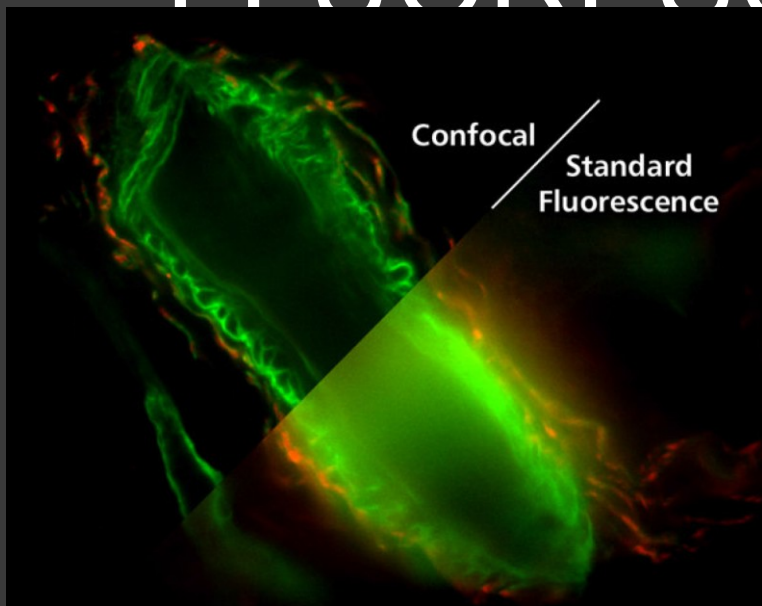


KONFOKÁLNÍ MIKROSKOP

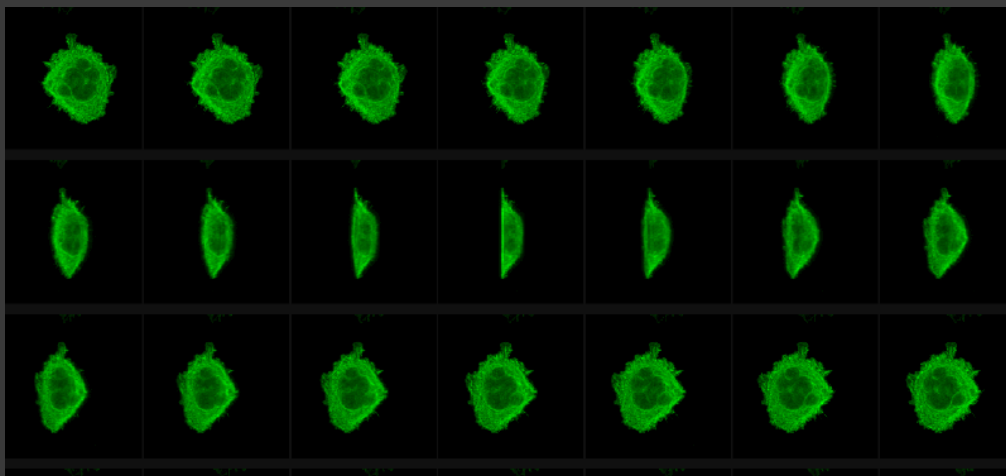
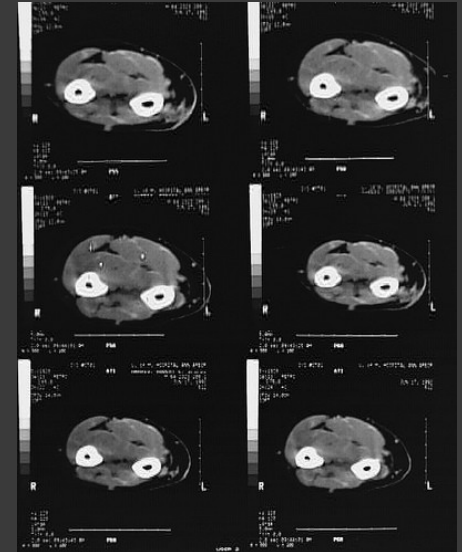
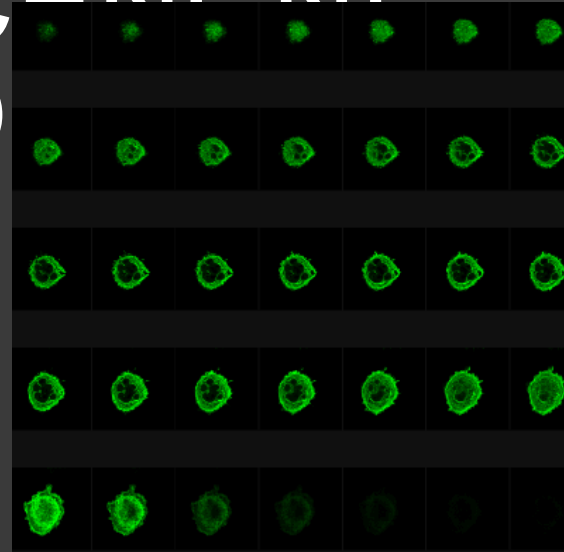
Leica



KONFOKÁLNÍ VS. FLUORESCENČNÍ



KONFOKÁLNÍ VS. FLUORESCENČNÍ



LIDSKÉ OKO

- 400-700 nm (380-760)

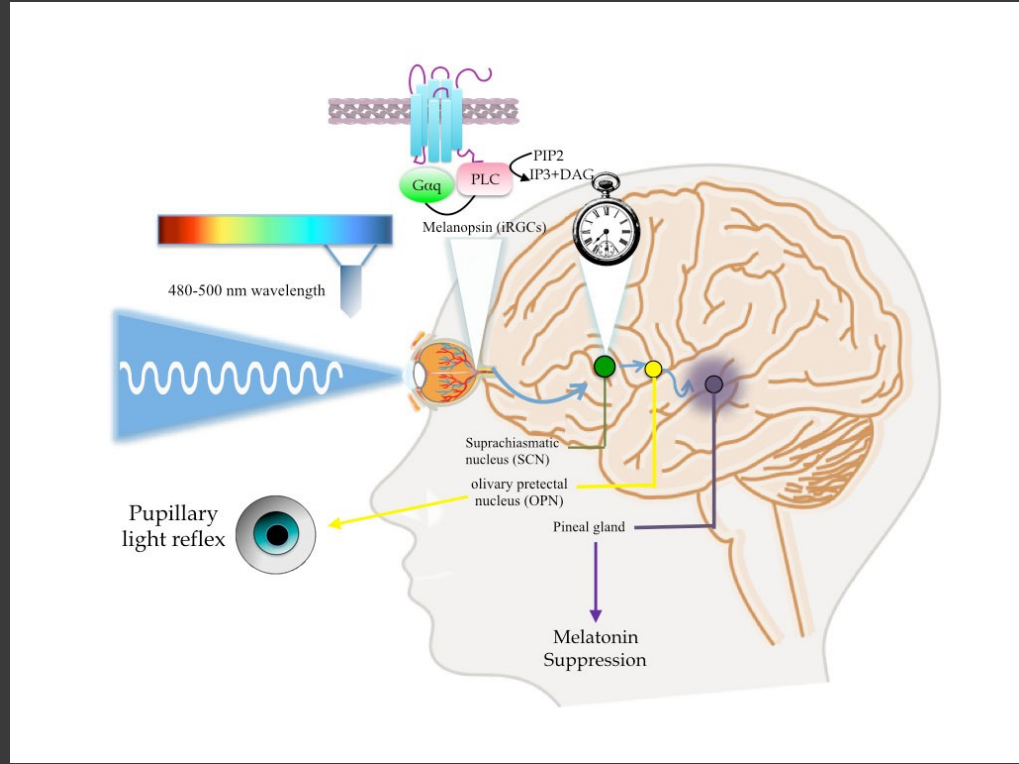
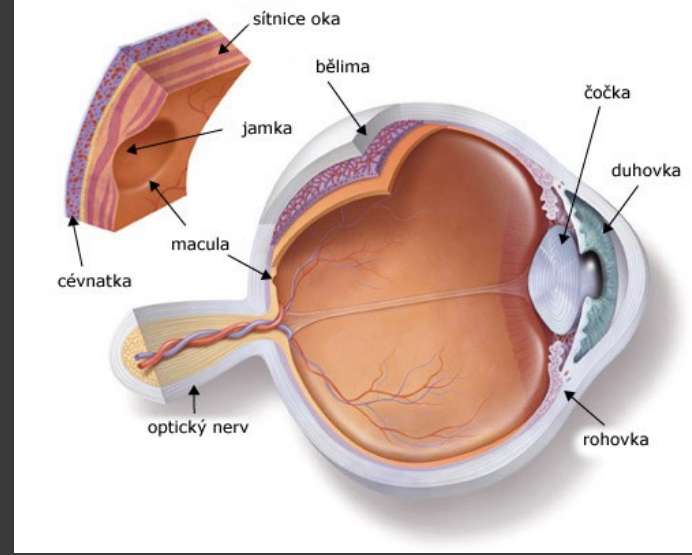
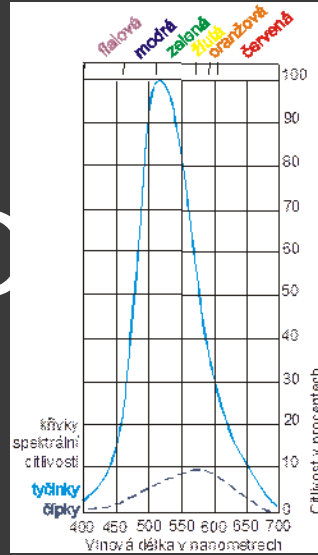
Tyčinky (černobílé)

- 500nm, 20x více než čípků

Čípky (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

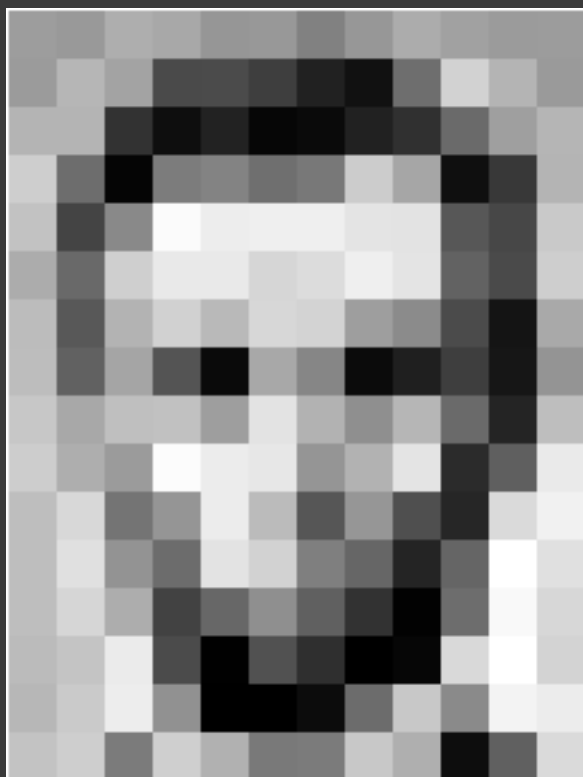
- 25snímků/s pohyb, zaostření 50 věcí/s, otisk prstu 40 parametrů – duhovka 256 parametrů



OBRAZ

2D tabulka pixelů

		COLUMNS			
		0	1	2	3
ROWS	0	0	25	50	75
	1	100	125	150	175
	2	200	225	250	255

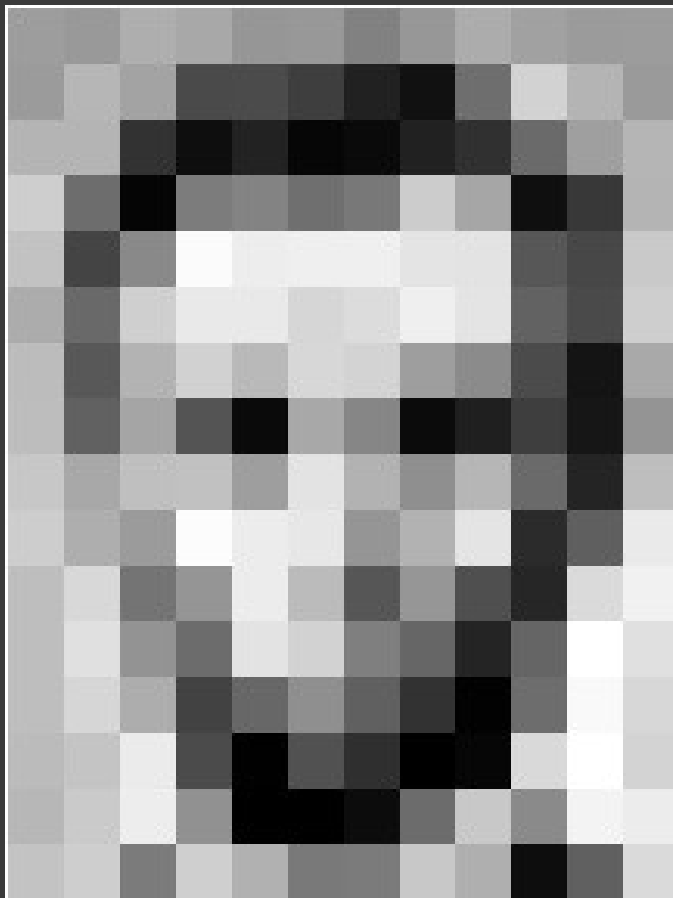
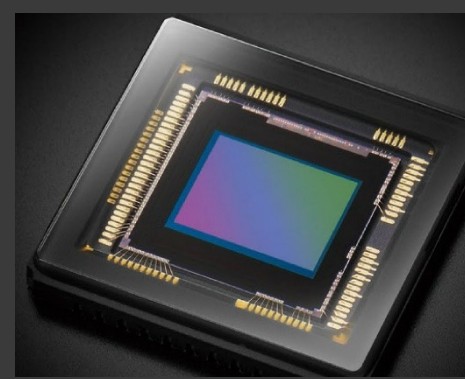


157	153	174	168	150	152	129	151	172	161	155	156
155	182	163	74	75	62	33	17	110	210	180	154
180	180	50	14	34	6	10	33	48	106	159	181
206	109	5	124	131	111	120	204	166	15	56	180
194	68	137	251	237	239	239	228	227	87	71	201
172	106	207	233	233	214	220	239	228	98	74	206
188	88	179	209	185	215	211	158	139	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

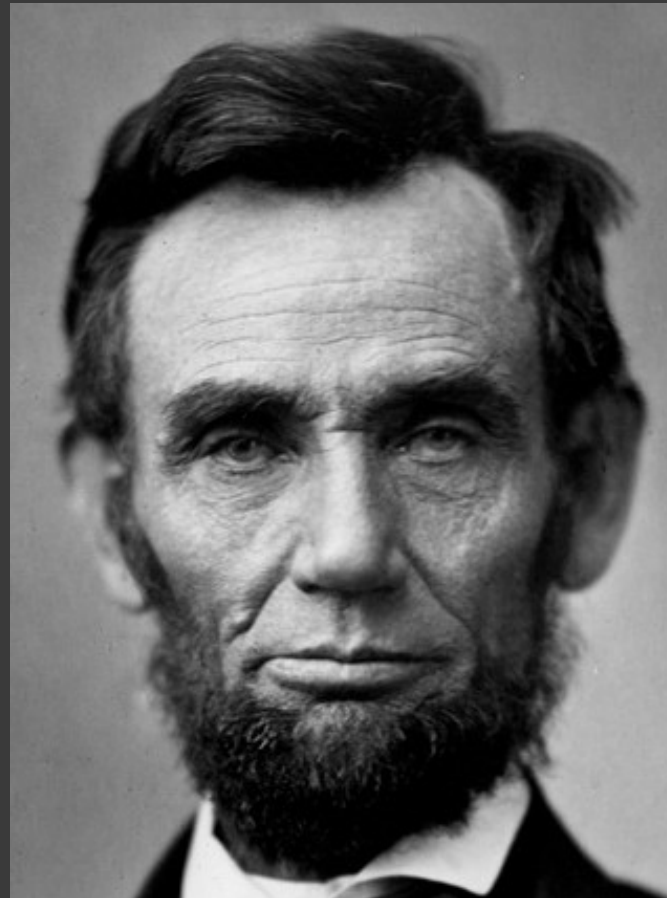
157	153	174	168	150	152	129	151	172	161	155	156
155	182	163	74	75	62	33	17	110	210	180	154
180	180	50	14	34	6	10	33	48	106	159	181
206	109	5	124	131	111	120	204	166	15	56	180
194	68	137	251	237	239	239	228	227	87	71	201
172	106	207	233	233	214	220	239	228	98	74	206
188	88	179	209	185	215	211	158	139	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

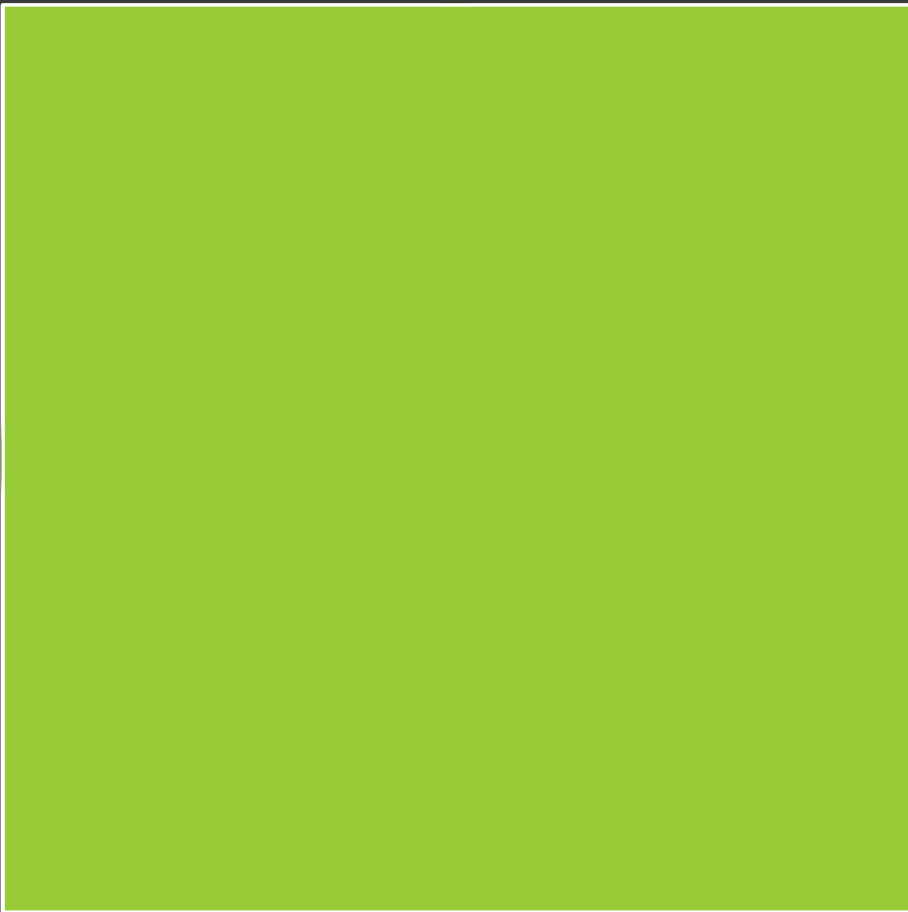
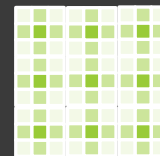


321 x 428 px

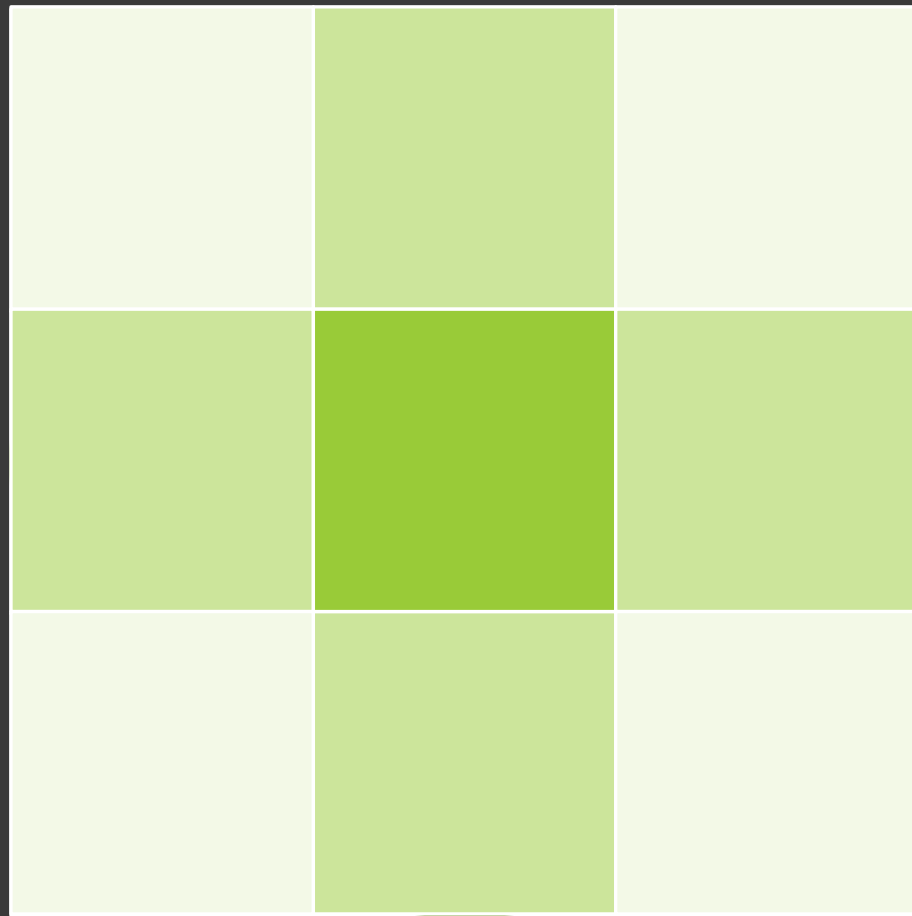
- počet pixelů
- CMOS/CCD čipy
- dpi – dots per inch
- tvar objektu vs. px
- zvětšení objektivu
- rozlišení objektivu



ROZLIŠENÍ



1 x 1 px



3 x 3 px

STUPNĚ ŠEDI

- Hladiny 150, 145, 140
- 30 stupňů šedi



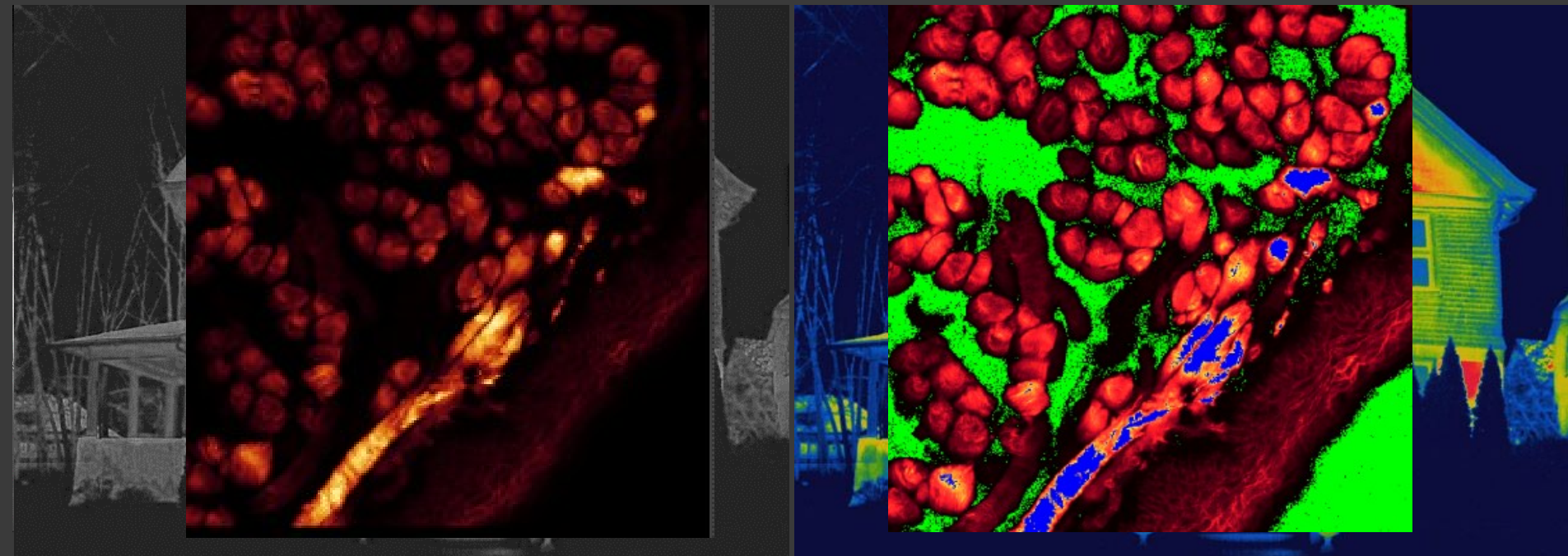
- okolo 900 stupňů šedi relativně

STUPNĚ ŠEDI



BAREVNÉ KÓDOVÁNÍ

- 10 milionů barev



ImageJ:
Analyze →
Histogram
Image → Type

HISTOGRAM – BITOVÁ HLOUBKA

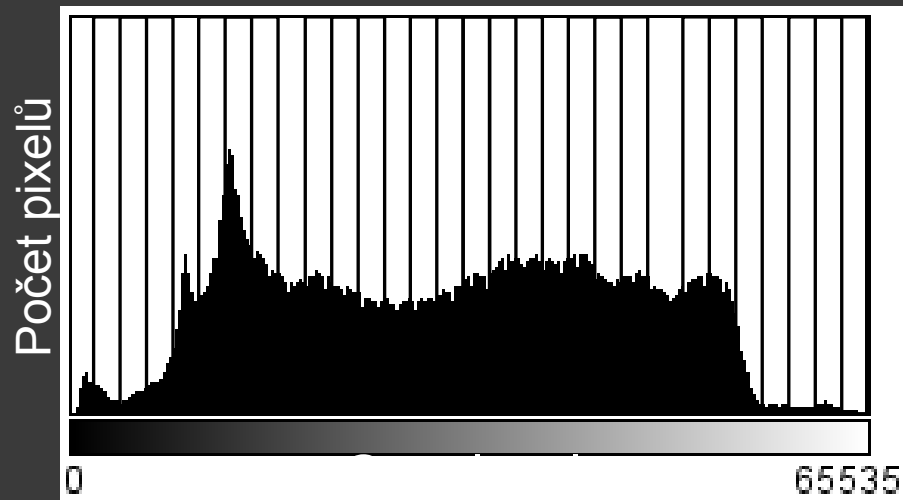
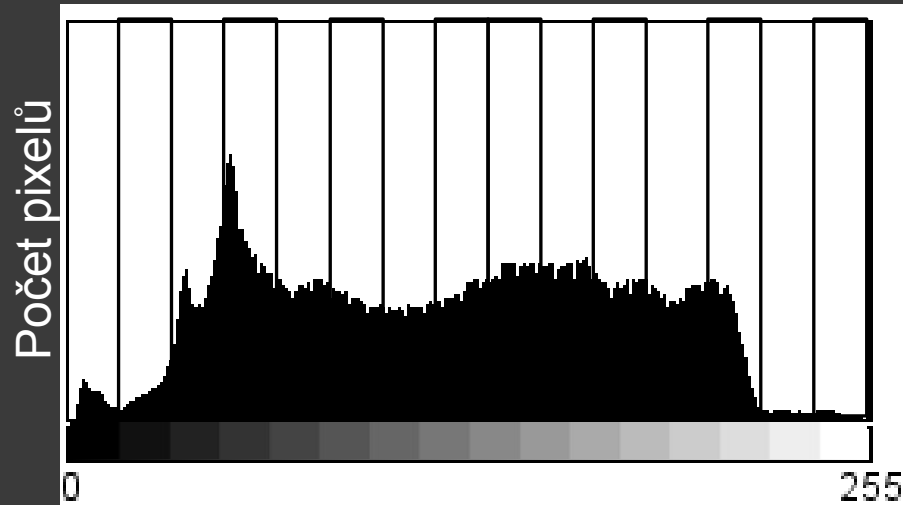
Originální obrázek

Histogram

Bitová
hloubka

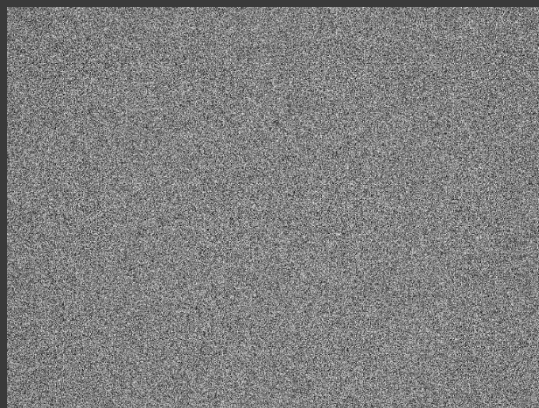
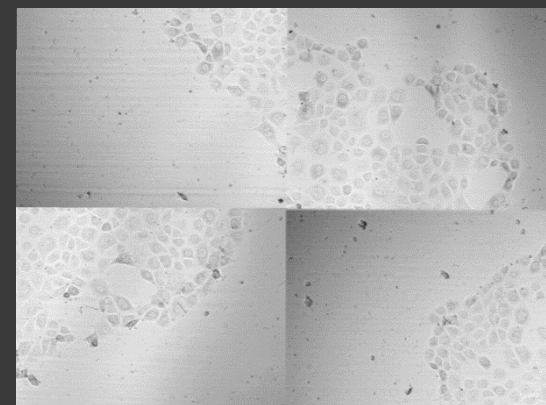
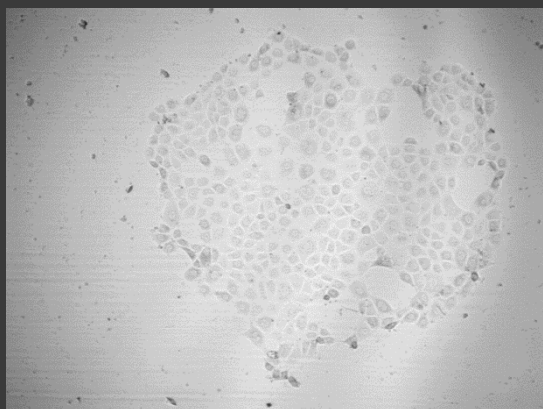
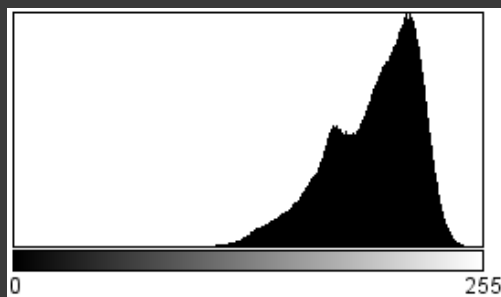
8 bit

16 bit



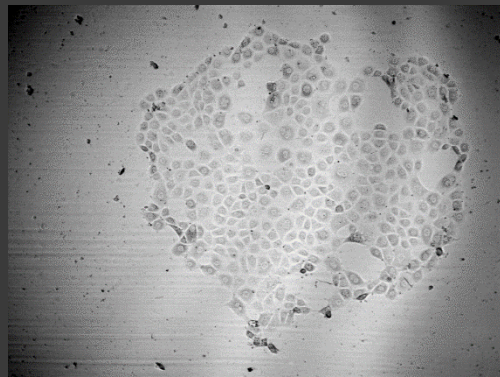
HISTOGRAM – REKONSTRUKCE OBRAZU

Histogram

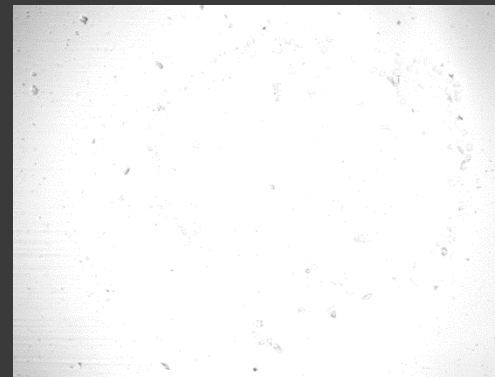
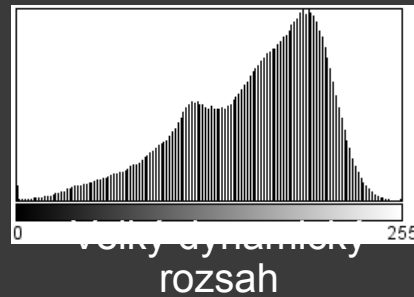


ImageJ:
Image → Adjust
→
Brightness/Contrast

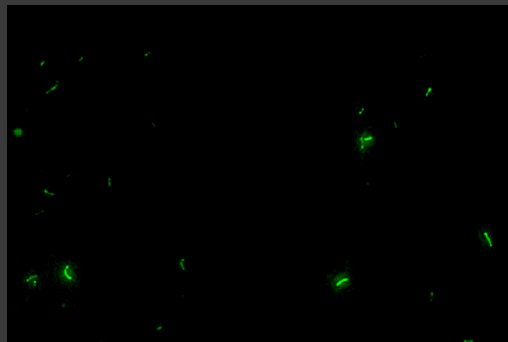
HISTOGRAM – DYNAMIC ROZSAH



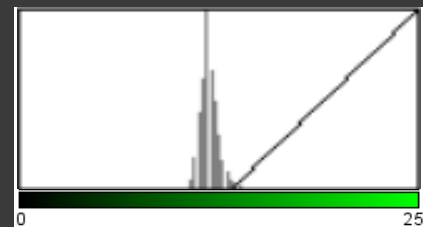
Histogram
Original



Krátká expozice



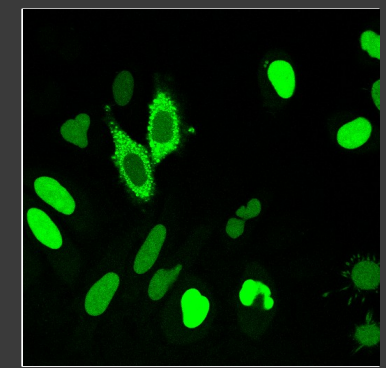
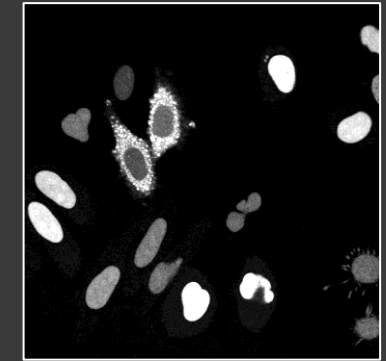
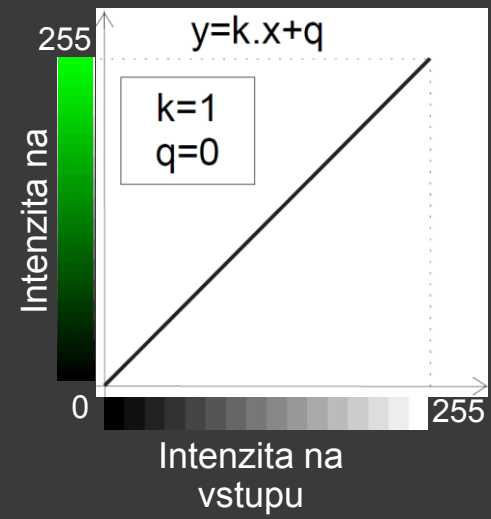
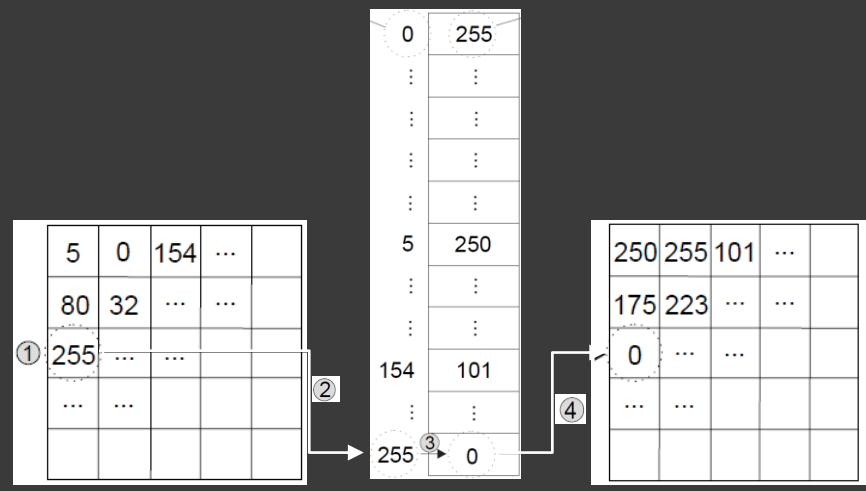
Odstření plozadí



ImageJ:
Image →
Lookup Tables
→ Invert
LUT/Green

HISTOGRAM & LUT – LOOKUP TABLE

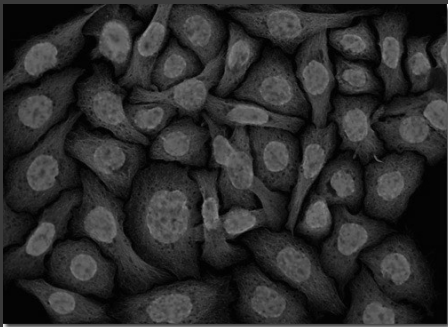
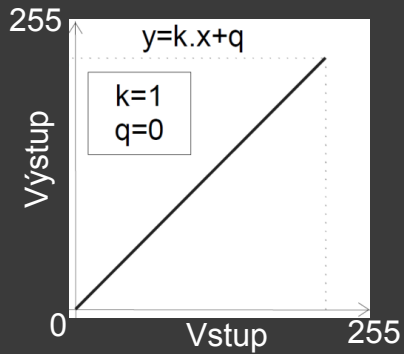
Jak LUT funguje



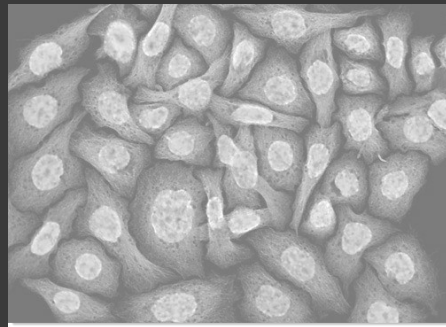
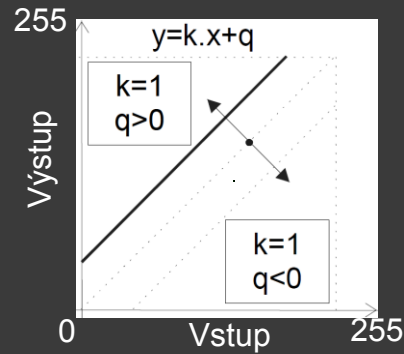
ImageJ:
Image → Adjust
→
Brightness/Contrast

HISTOGRAM & LUT – PŘÍKLADY

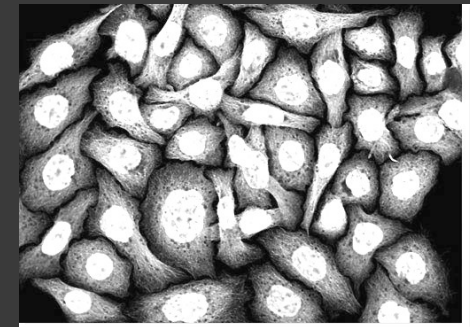
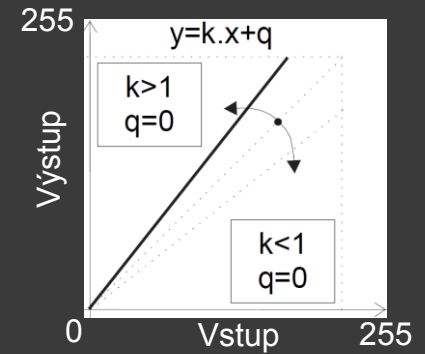
Žádná změna



Jas

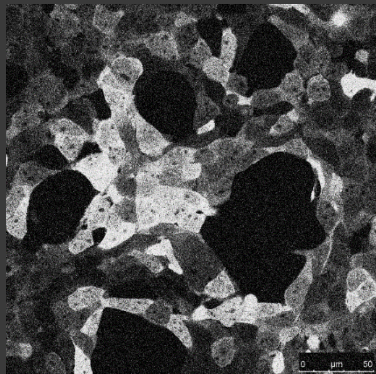


Kontrast

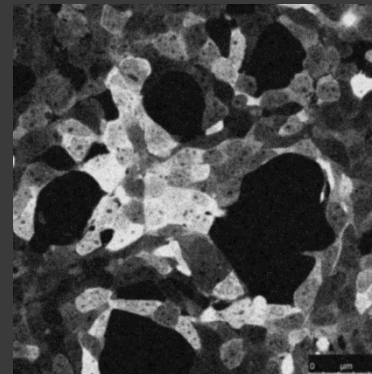


ImageJ:
Process → Filters
→ Gaussian
Blur/Median

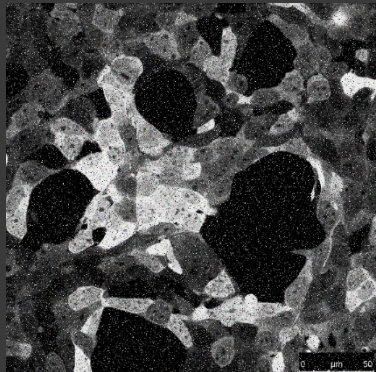
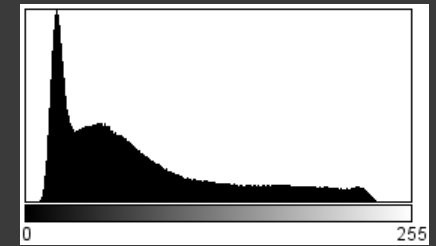
HISTOGRAM - ŠUM



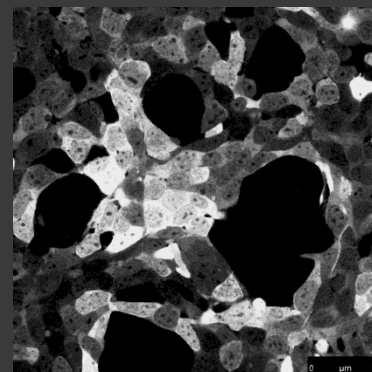
Gaussovský



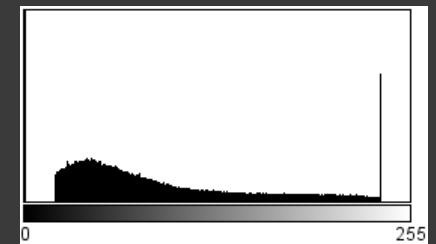
Blur filtering



Sůl & Pepř



Median filtering



DATOVÉ FORMÁTY

Rastrové obrazy

Tvar reprezentovaný maticí pixelů

Pixelace

Fotorealistické obrazy

Typické formáty: JPEG, GIF, TIFF, PNG

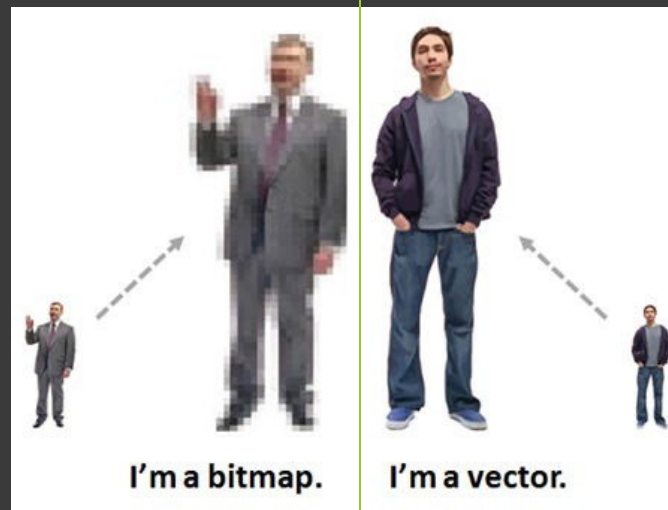
Vektorové obrazy

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)

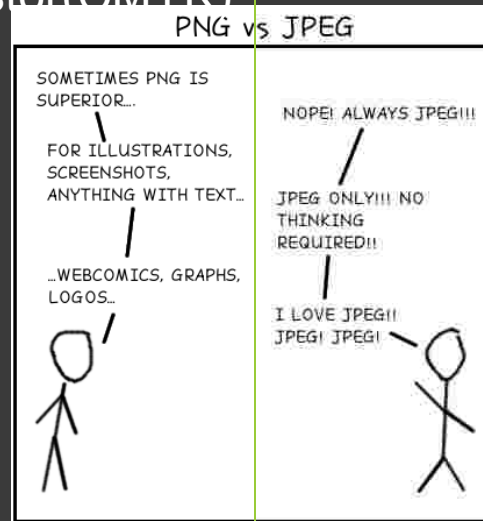
Joint Photographic Expert Group (JPEG)

Fotky a realistické obrazy

Ztrátová komprese

Jemné, pozvolné přechody

Nepodporuje transparentnost

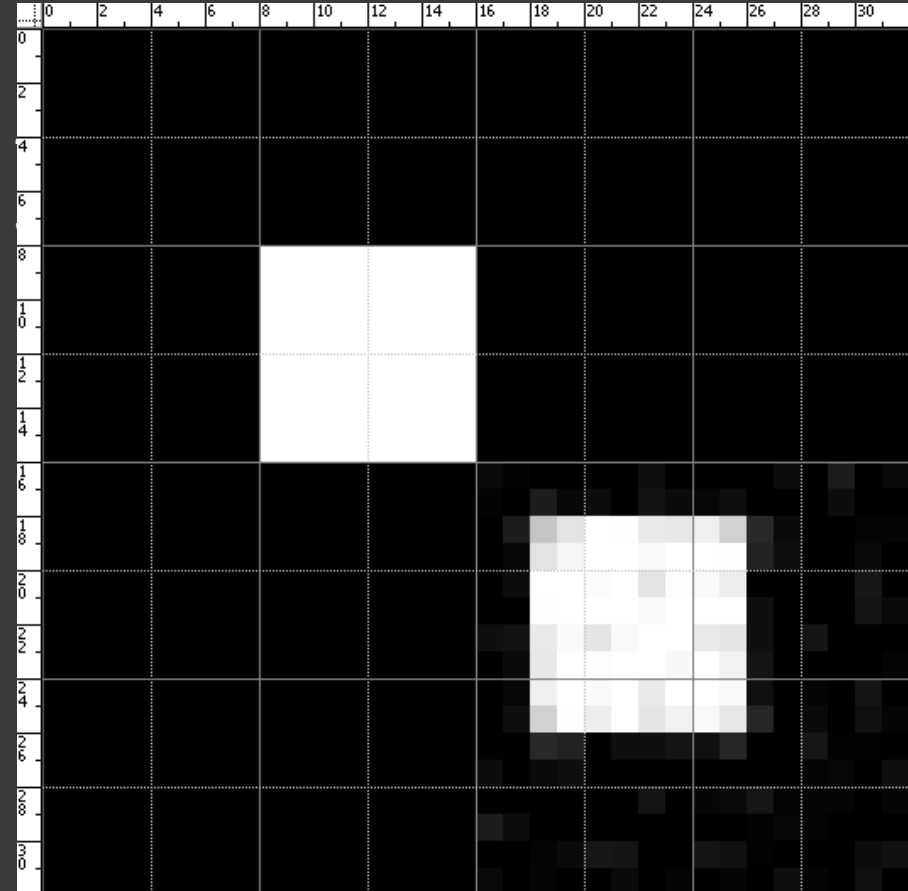


ImageJ:
File → Save As →
PNG/Jpeg

DATOVÉ FORMÁTY – RASTROVÁ GRAFIKA



Joint Photographic Expert Group (JPEG)



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



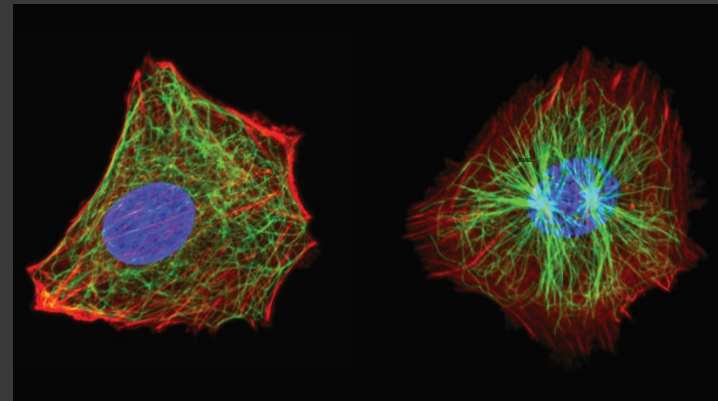
Tagged Image File Format (TIFF)

Fotky, grafika

Možnost bezeztrátové komprese

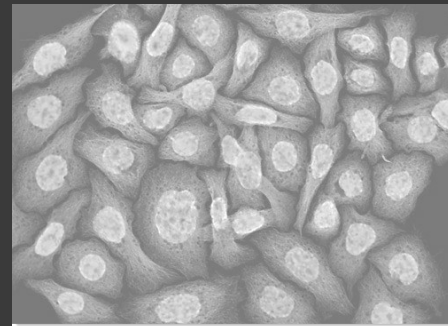
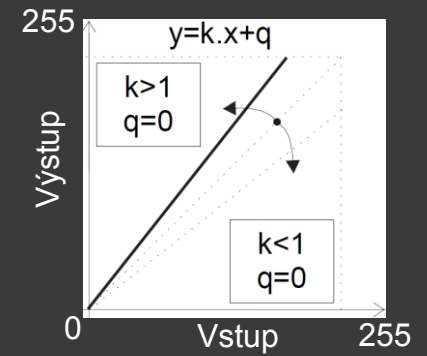
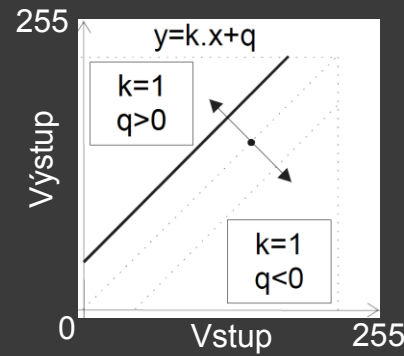
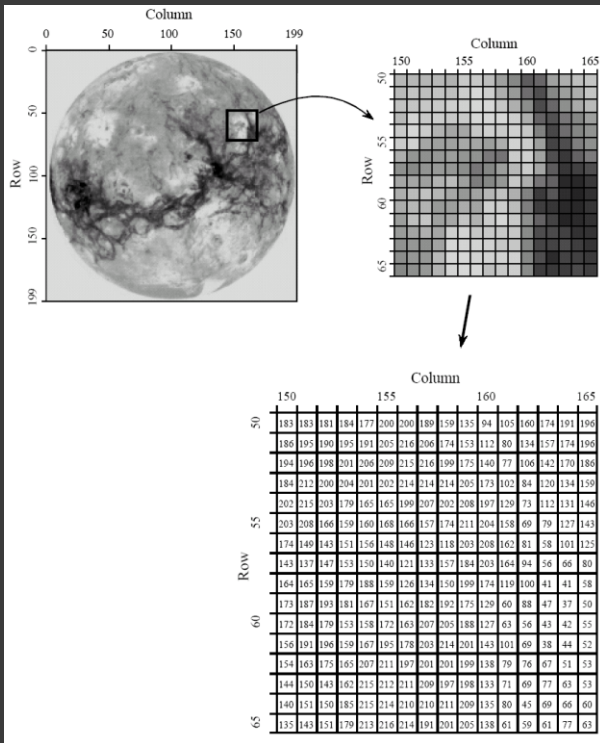
Profesionální, široce podporovaný

Různé barevné prostory, jakékoli rozlišení
a barvy

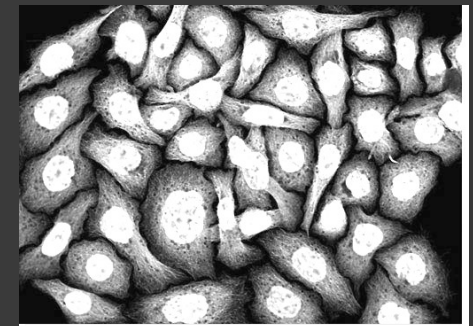


ImageJ:
Image → Adjust
→
Brightness/Contrast

ZPRACOVÁNÍ – BODOVÉ TRANSFORMACE

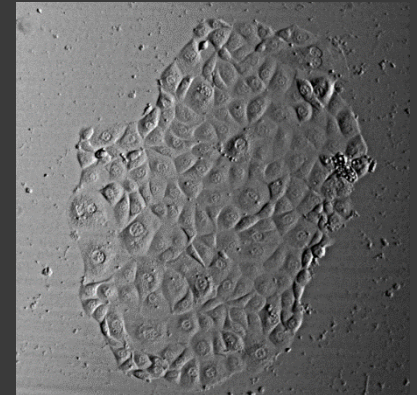
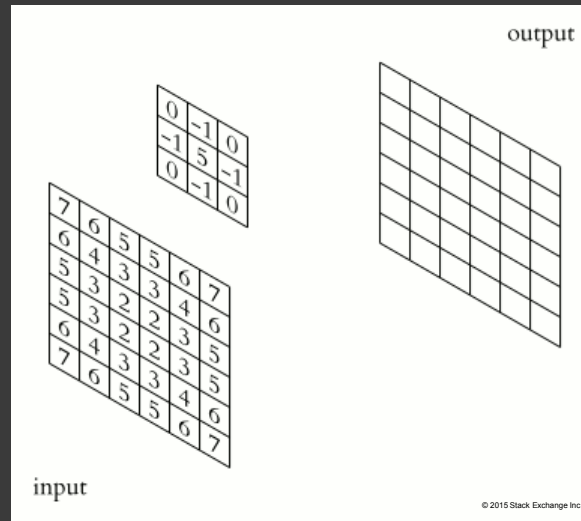
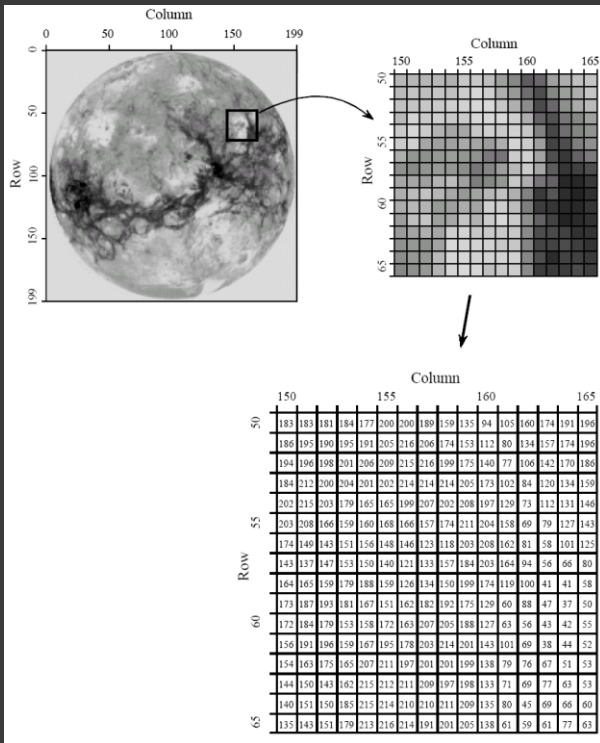


✖ 30



FILTROVÁNÍ – LOKÁLNÍ ZMĚNY

ImageJ:
Process →
Sharpen/Filters →
Unsharp Mask



ImageJ:

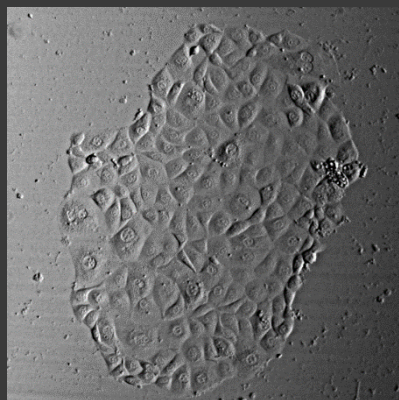
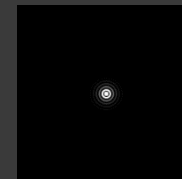
Process →
Sharpen/Smooth/
Find Edges
Plugins → Parallel
Iterative
Deconvolution

FILTROVÁNÍ – PŘÍKLADY

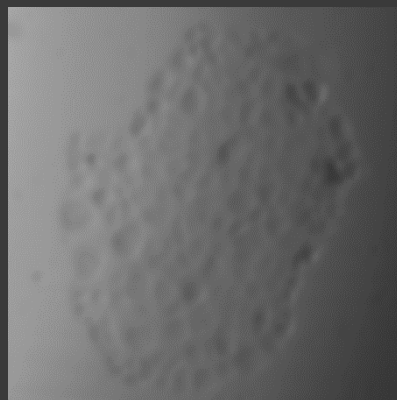
0	0	0	0	0
0	0	-1	0	0
0	-1	5	-1	0
0	0	-1	0	0
0	0	0	0	0

0	0	0	0	0	0
0	1	1	1	0	
0	1	1	1	0	
0	1	1	1	0	
0	0	0	0	0	

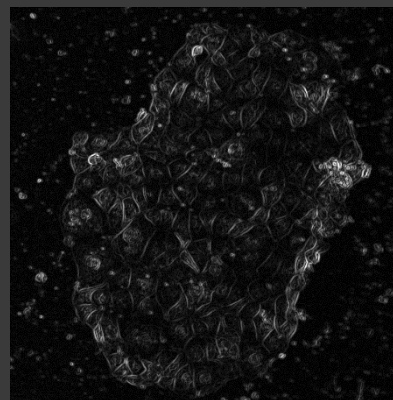
	0	1	0	
	1	-4	1	
	0	1	0	



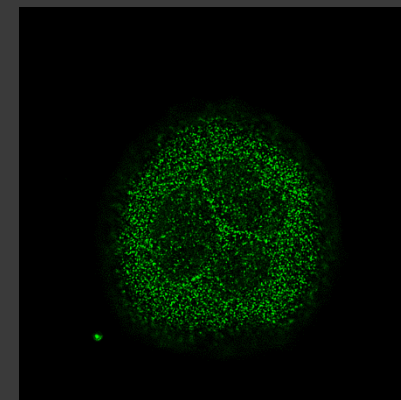
doostření



rozmazání



detekce hran

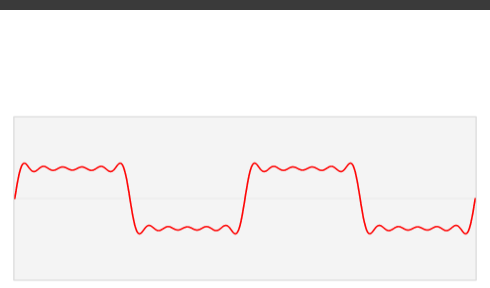
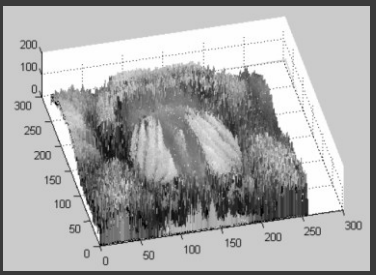
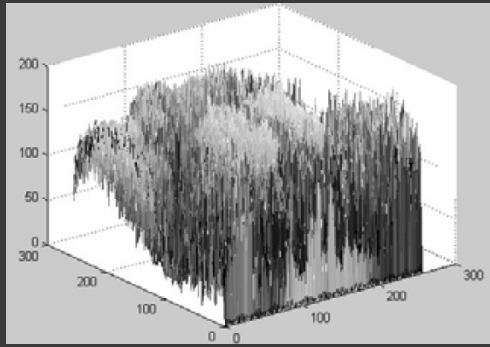


dekonvoluce

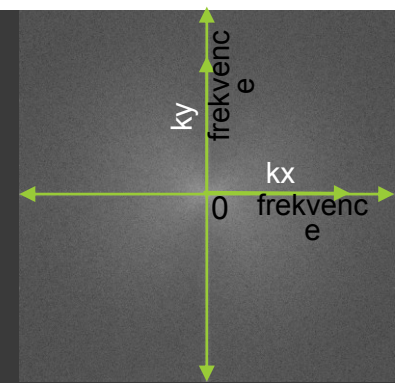
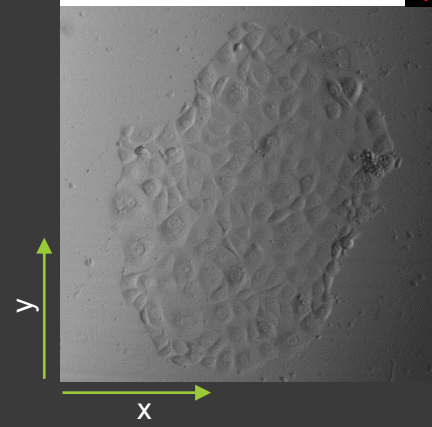
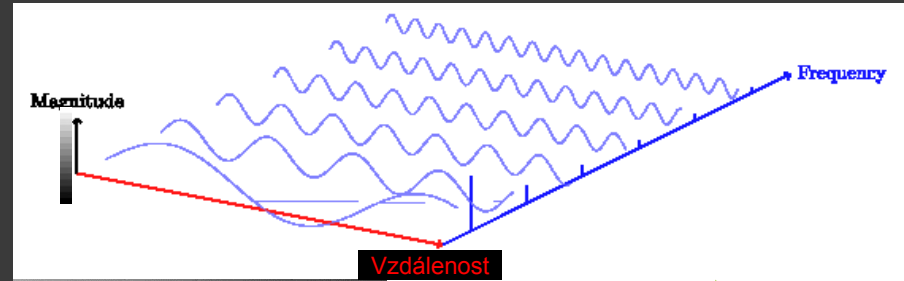
ImageJ:
Process → FFT →
FFT

FILTROVÁNÍ – GLOBÁLNÍ TRANSFORMACE

Fast Fourier Transformation

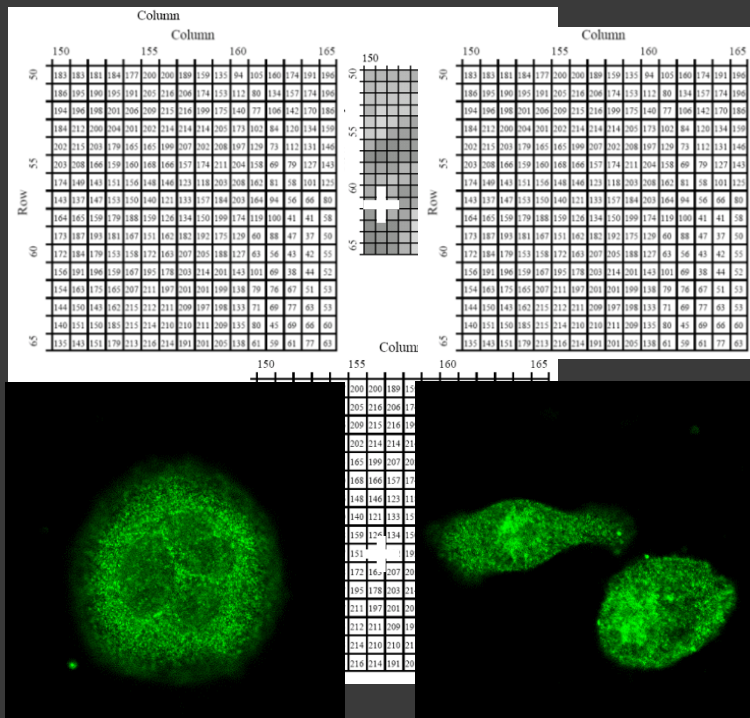


Konvoluce = Násobení

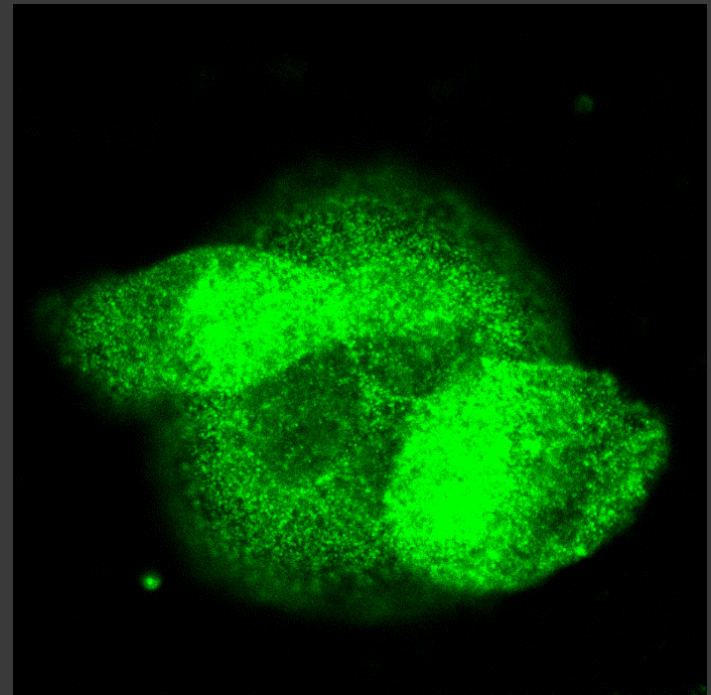


OBRAZOVÁ ARITMETIKA

Sčítání



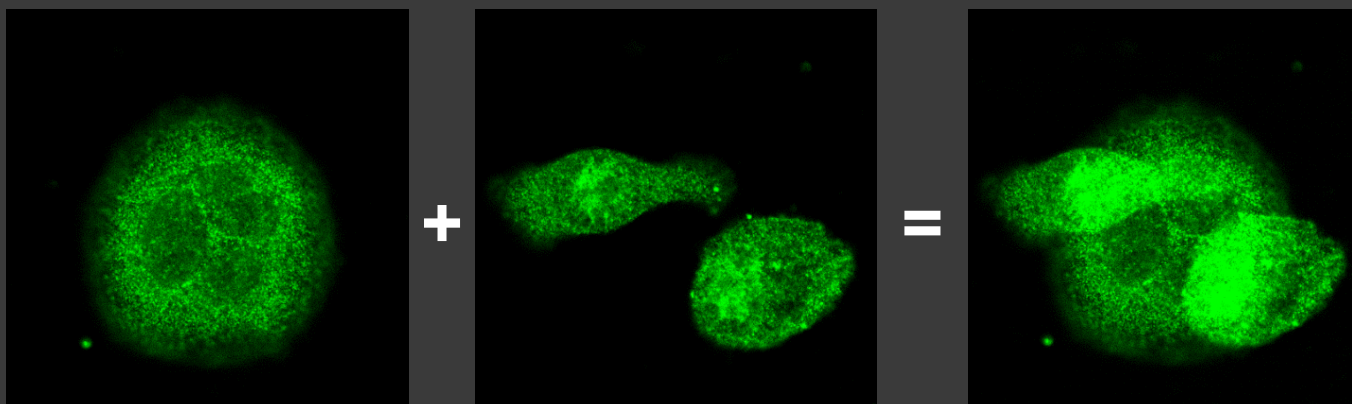
==



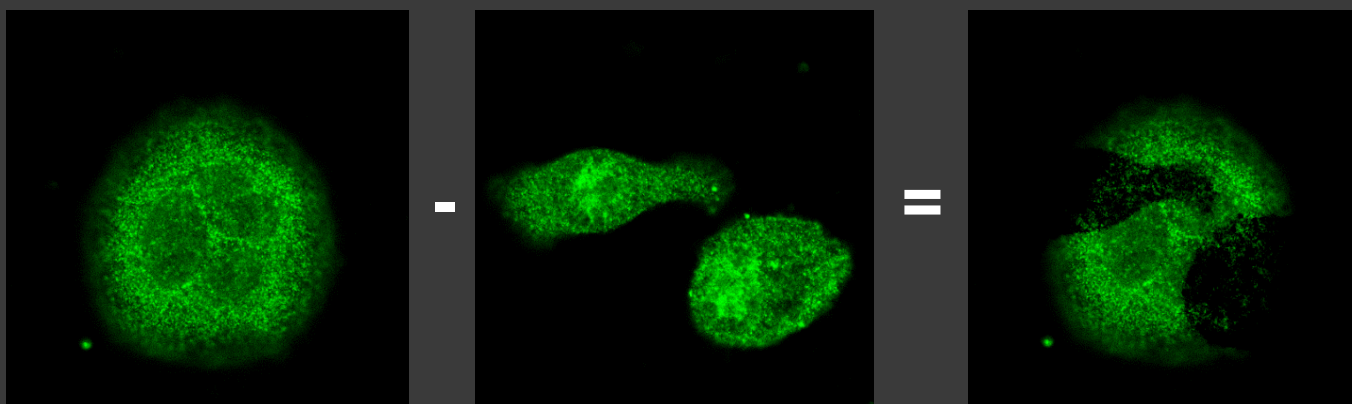
==

OBRAZOVÁ ARITMETIKA

Sčítání

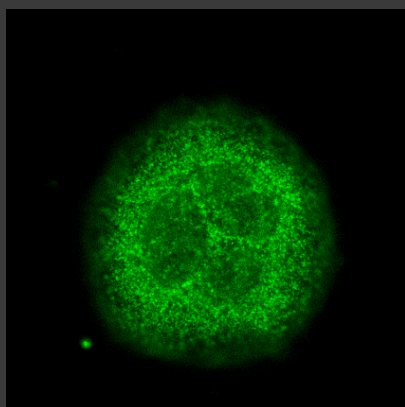


Odčítání

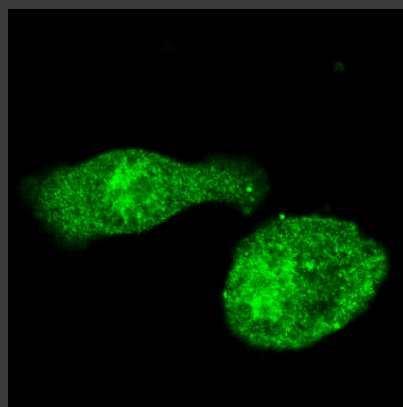


OBRAZOVÁ ARITMETIKA

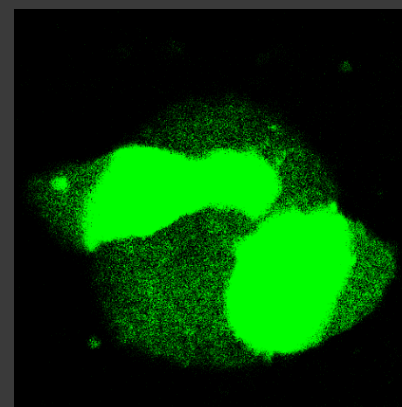
Násobení



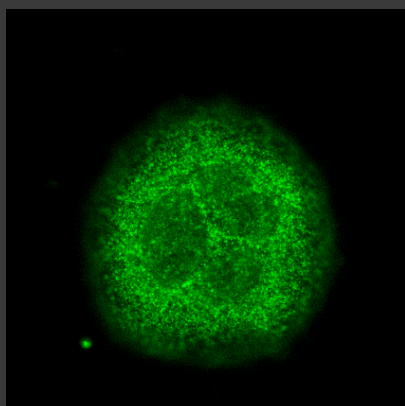
x



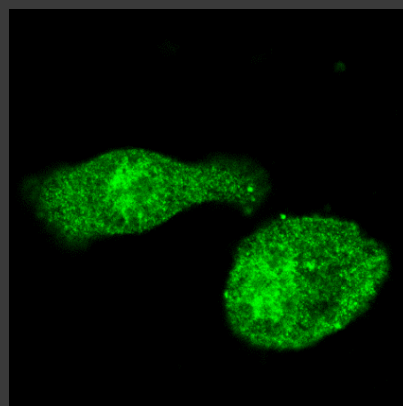
=



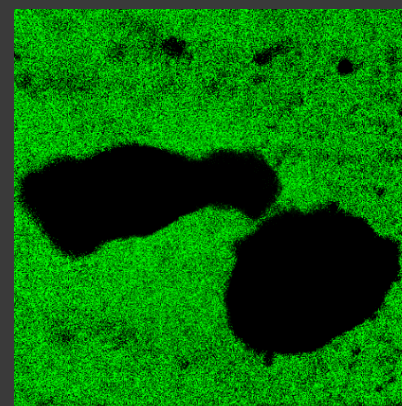
Dělení



/

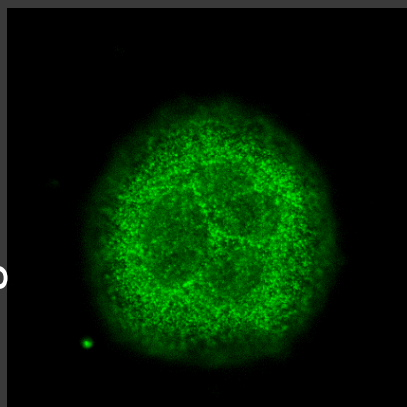


=

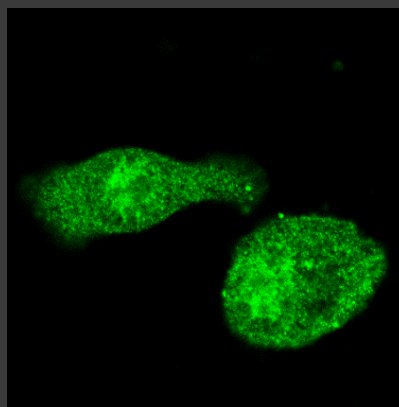


OBRAZOVÁ ARITMETIKA

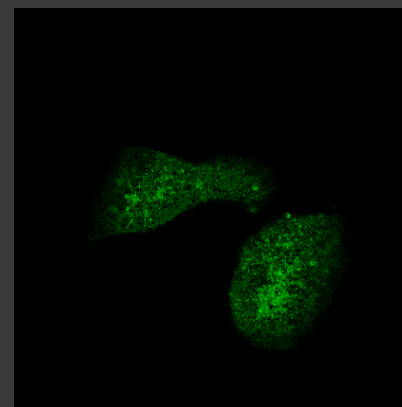
Logické AND



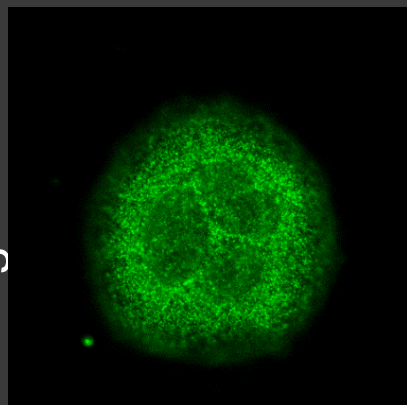
AND



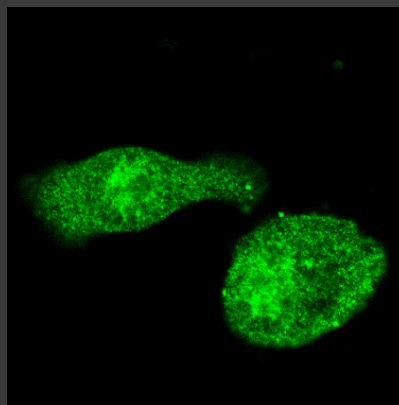
==



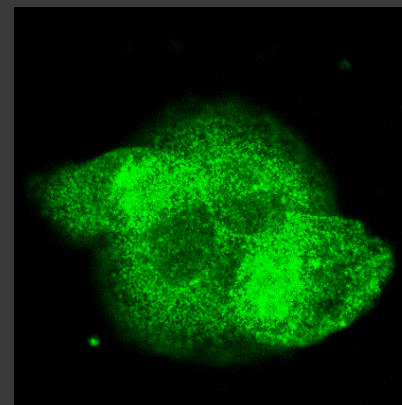
Logické OR



OR



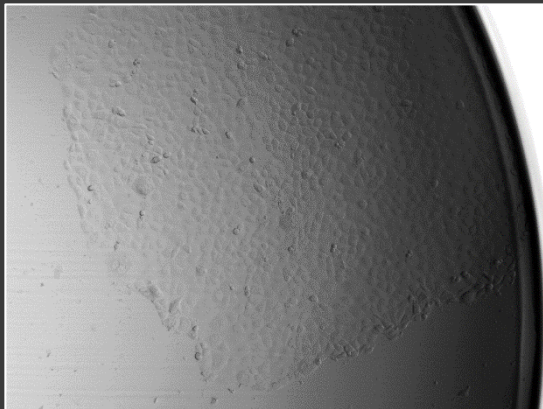
==



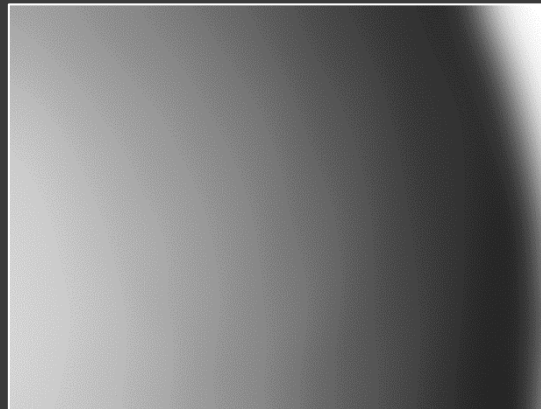
OBRAZOVÁ ARITMETIKA – PŘÍKLADY

ImageJ:
Process → Filters
→ Gaussian Blur
→ Save As
Process → Image
Calculator

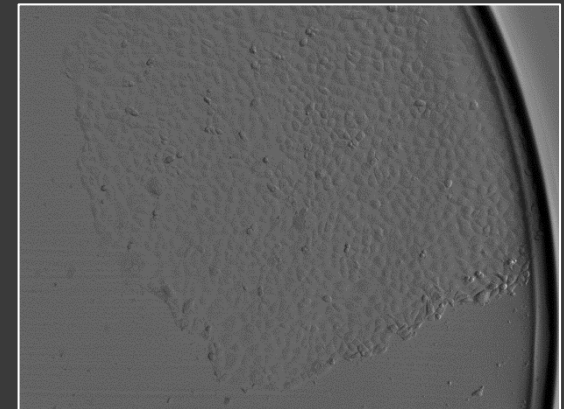
Flat Field korekce



/



=

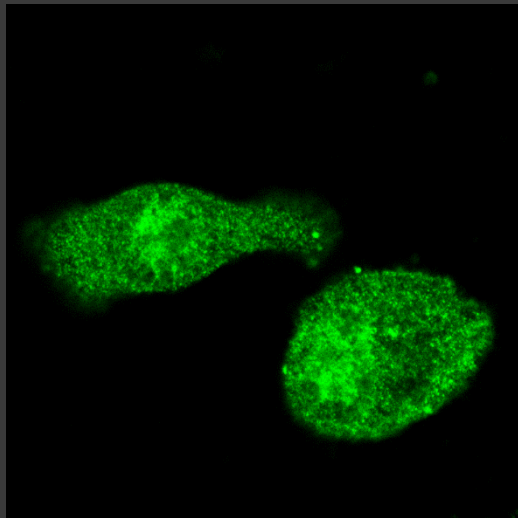


Gaussian blur

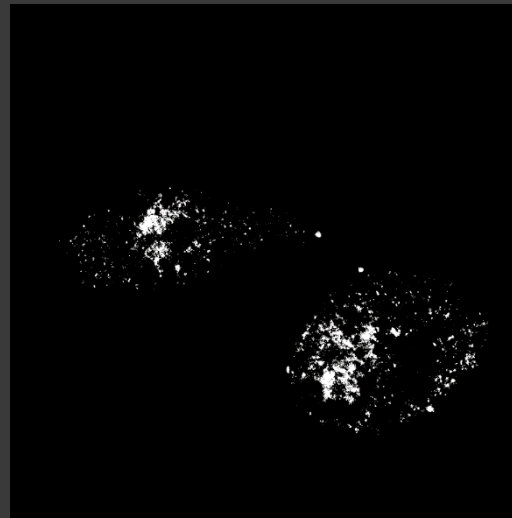
OBRAZOVÁ ARITMETIKA – PŘÍKLADY

ImageJ:
Image → Adjust
→ Threshold →
Save As
Process → Image
Calculator

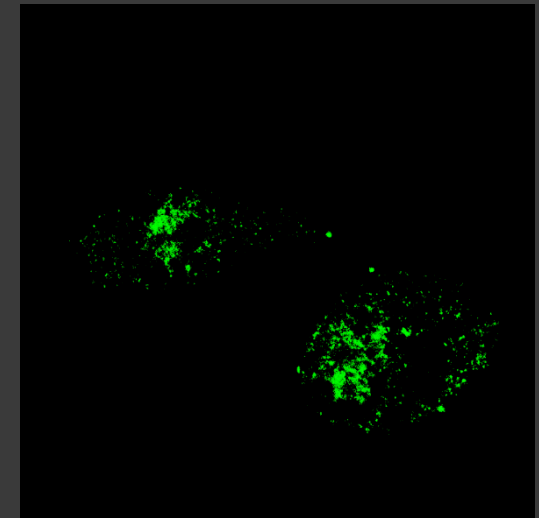
Segmentace



AND



=

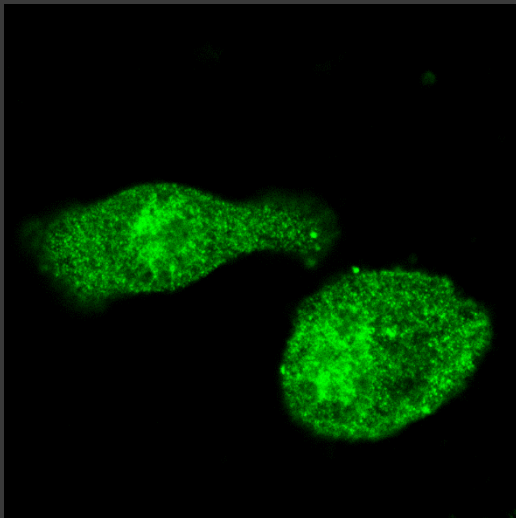


Maska

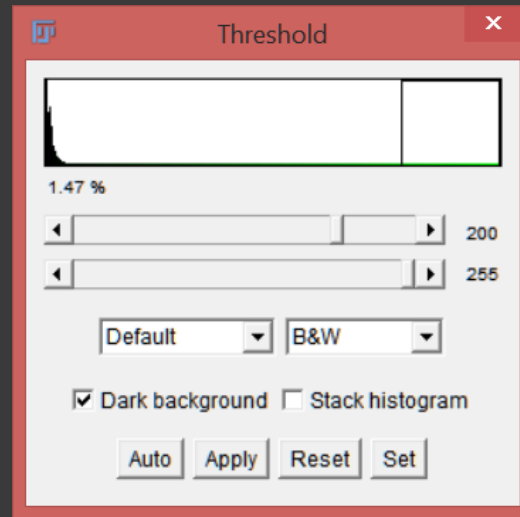
ImageJ:
Image → Adjust
→ Threshold

SEGMENTACE – MANUÁLNÍ PRAHOVÁNÍ TRESHOLDING

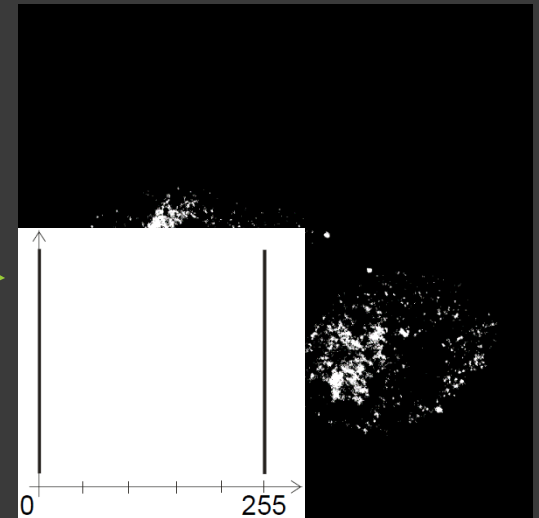
Originální obraz



Prahování



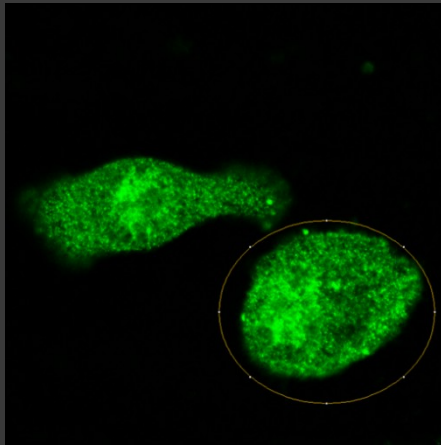
Maska



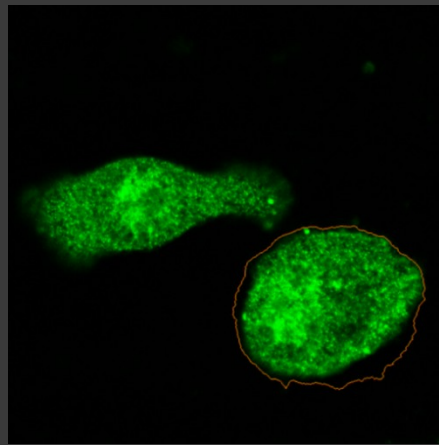
ImageJ:
Plugins →
Segmentation →
Level Sets

SEGMENTACE – POLOAUTOMATICKÁ

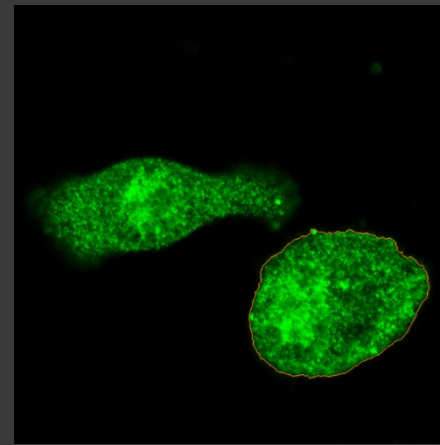
Start - ROI



Level sets



Konec

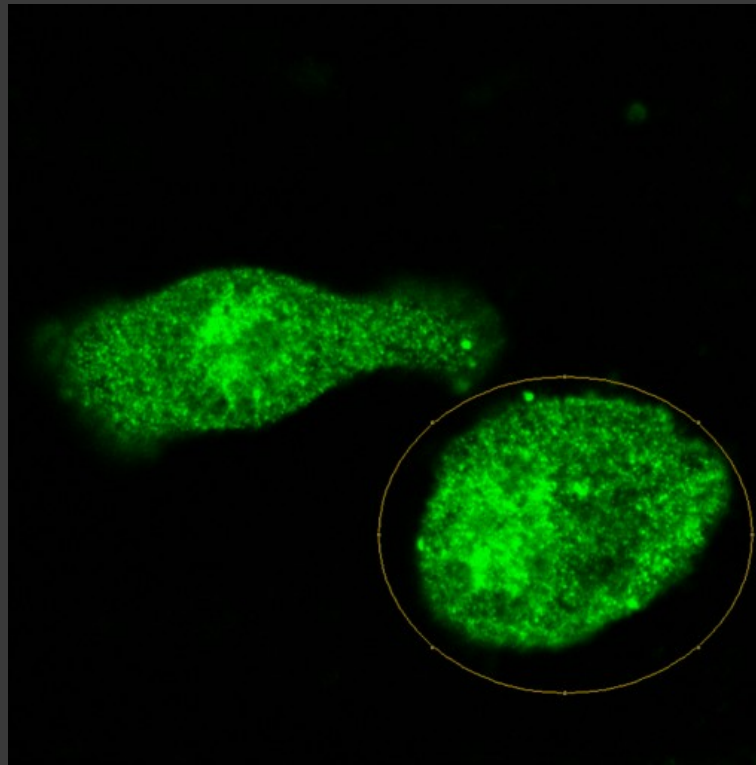


Maska



ImageJ:
Plugins →
Segmentation →
Level Sets

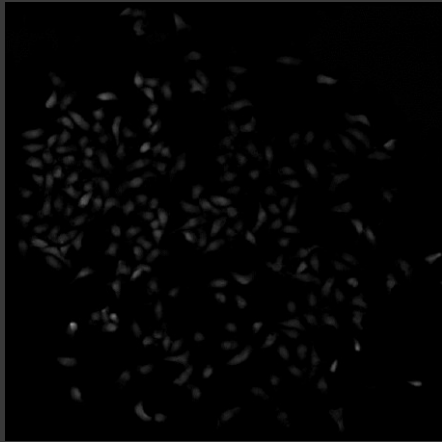
SEGMENTACE – POLOAUTOMATICKÁ



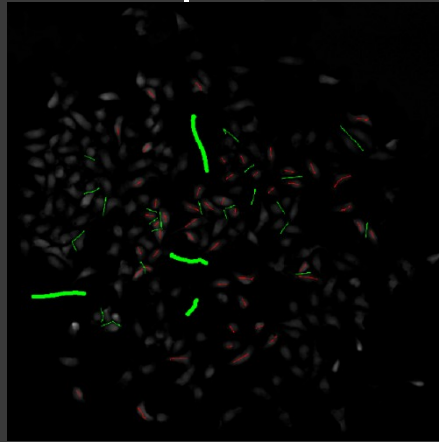
SEGMENTACE – STROJOVÉ UČENÍ

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

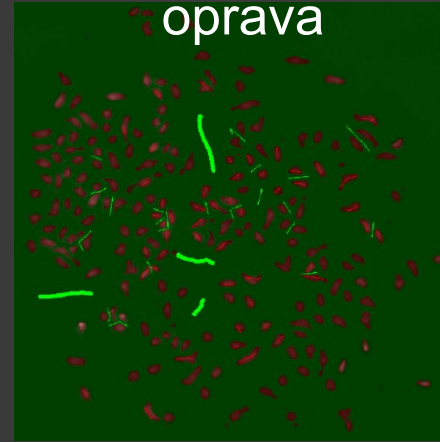
Originální obraz



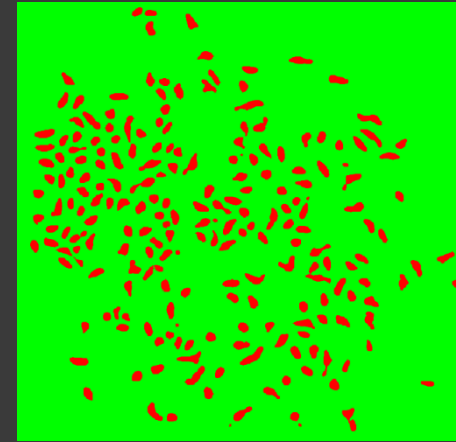
Učení



Kontrola a
oprava



Maska



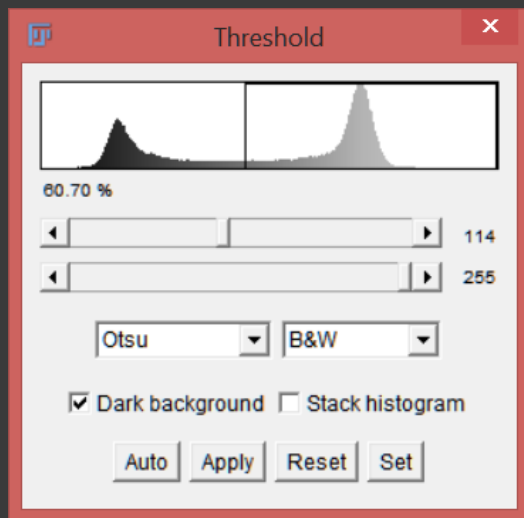
SEGMENTACE – AUTOMATIZOVANÁ

ImageJ:
Image → Adjust
→ Threshold →
Otsu

Originální obraz

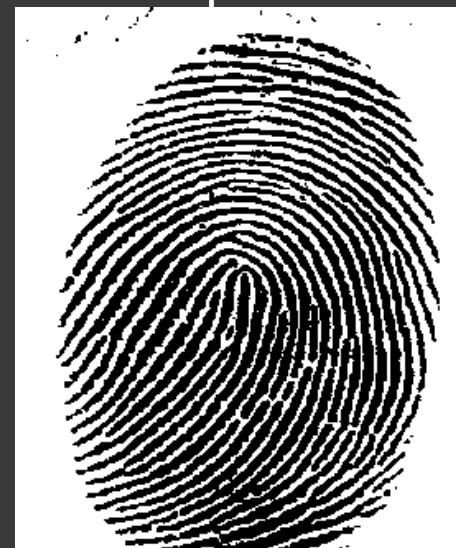


Otsu - Klastrování



Intra-class variance
minimal

Segmentovaný



ZMĚNA MORFOLOGIE MASKY

ImageJ:
Process → Binary
→ Make Binary
Process → Binary
→
Dilate/Erode/Open
/Close

Roztažení



+ Structuring
element



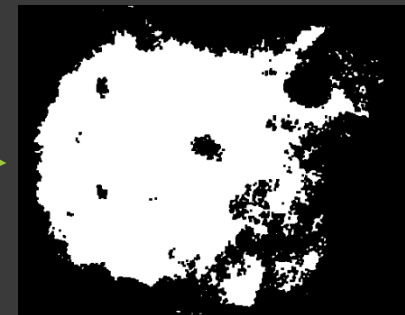
Otevření



Eroze



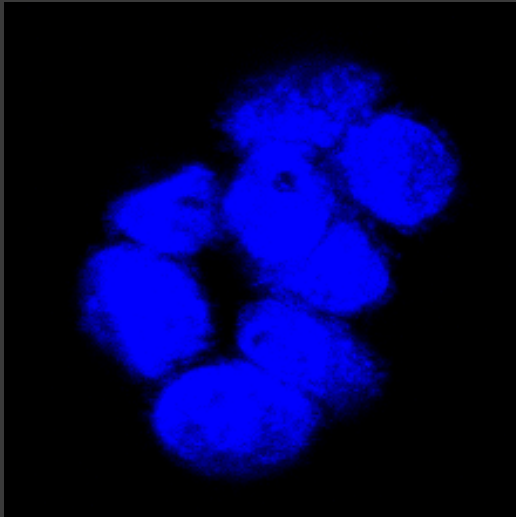
Zavření



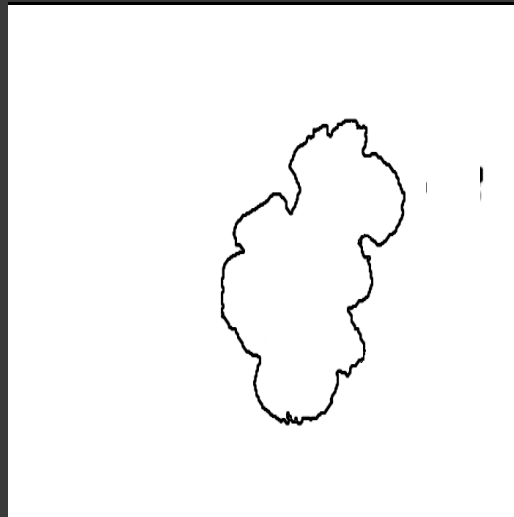
ZMĚNA MORFOLOGIE MASKY

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

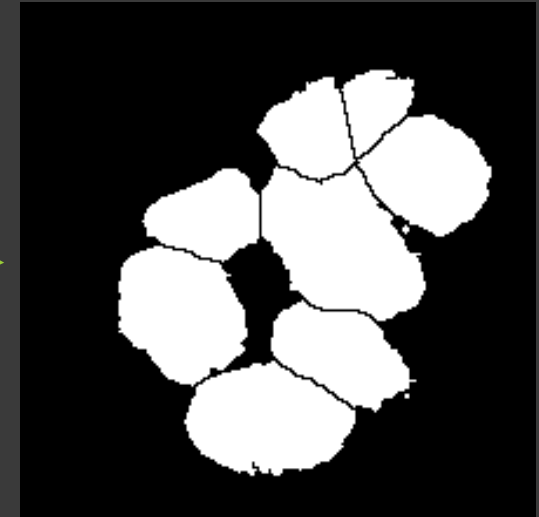
Originální obrazy



Prahování



Watershed

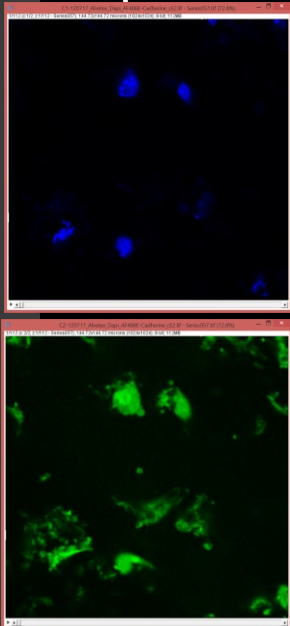


Voda stéká do údolí

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

SEGMENTACE – 3D MODELOVÁNÍ

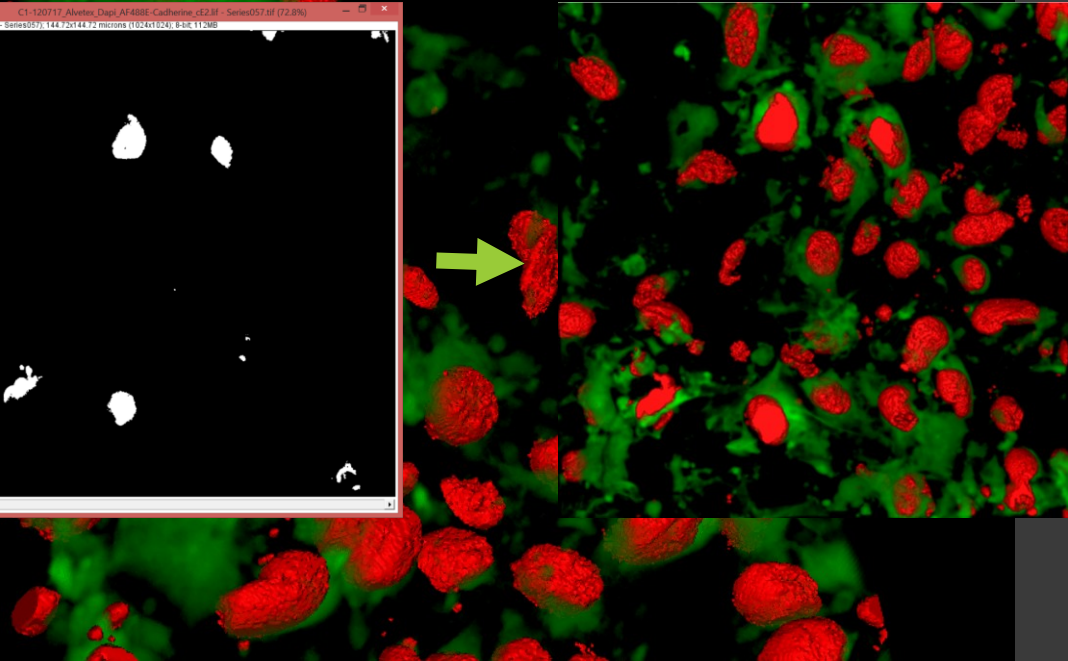
Originální



Oprahovaná jádra



3D projekce



ImageJ:
Image →
Properties
Analyze →
Analyze Particles

OBRAZOVÁ ANALÝZA - STEREOLOGIE

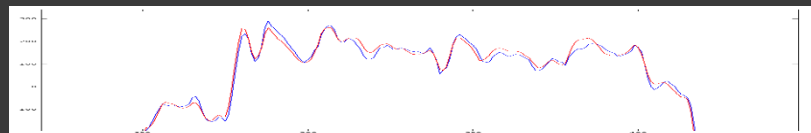
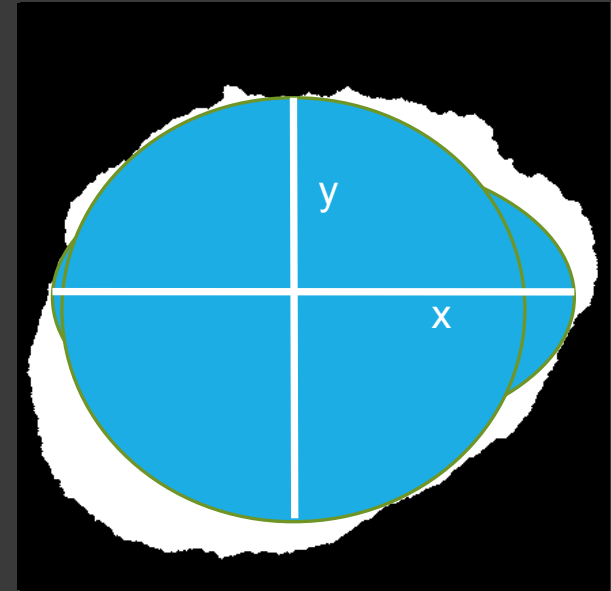
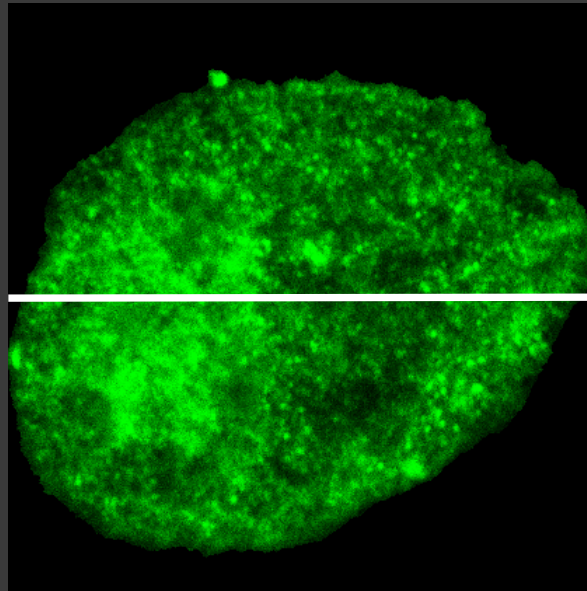
Velikost pixelu

Plocha

Intenzita (bitová hl.)

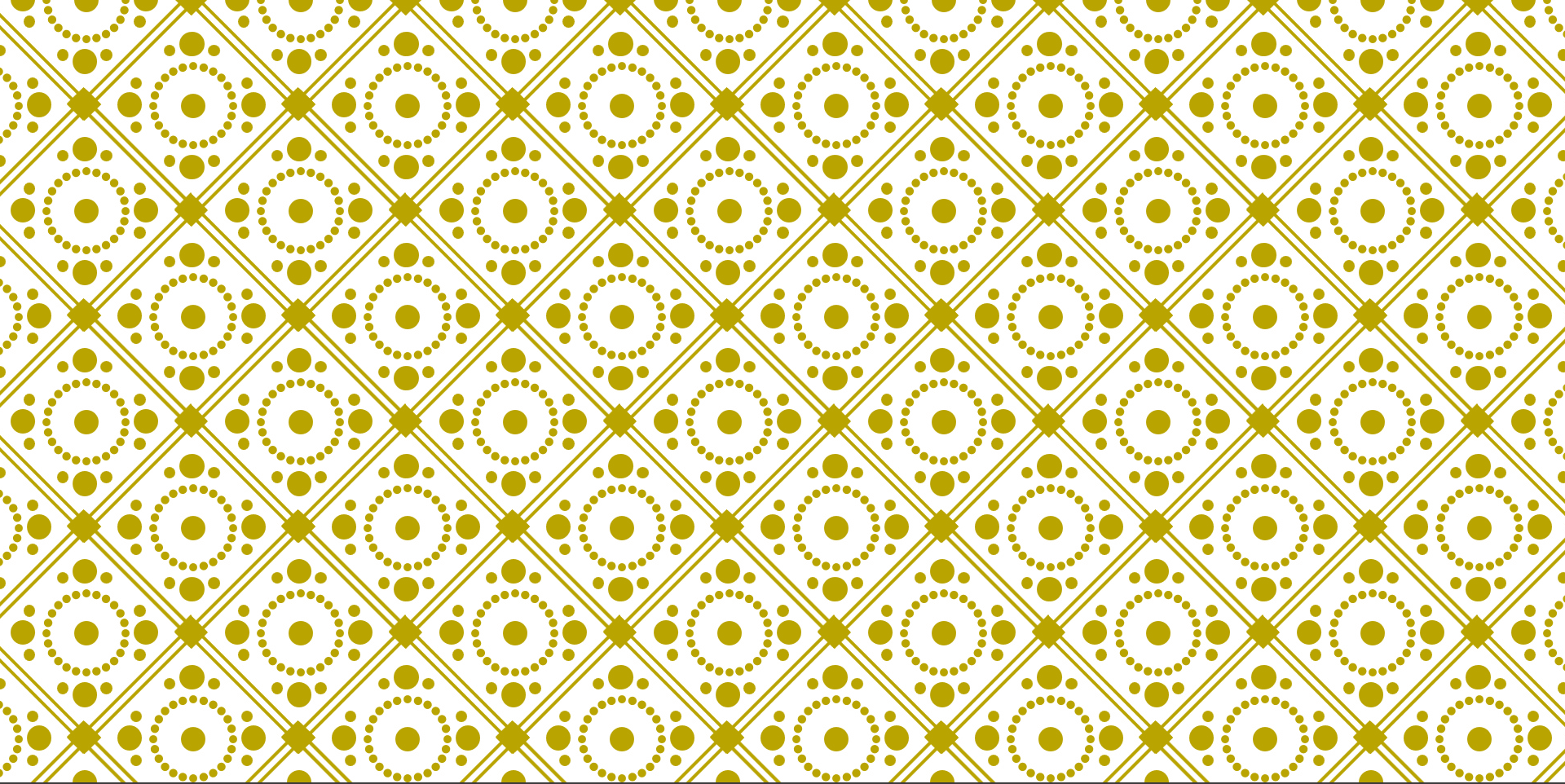
Textura

Morfologie



PROBLÉMY

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

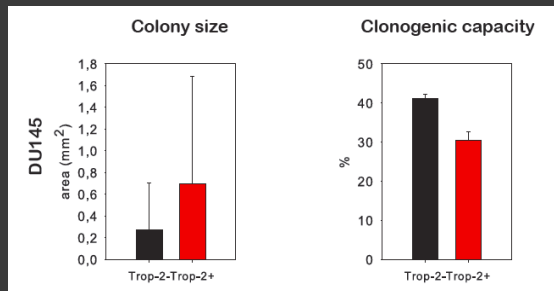
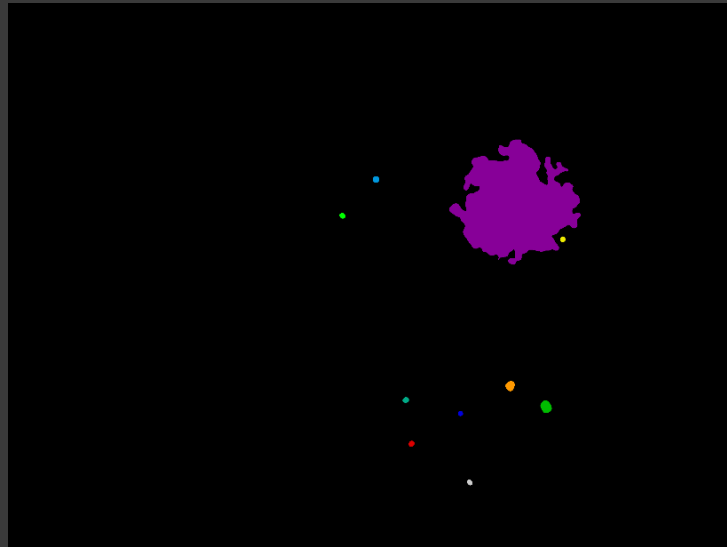


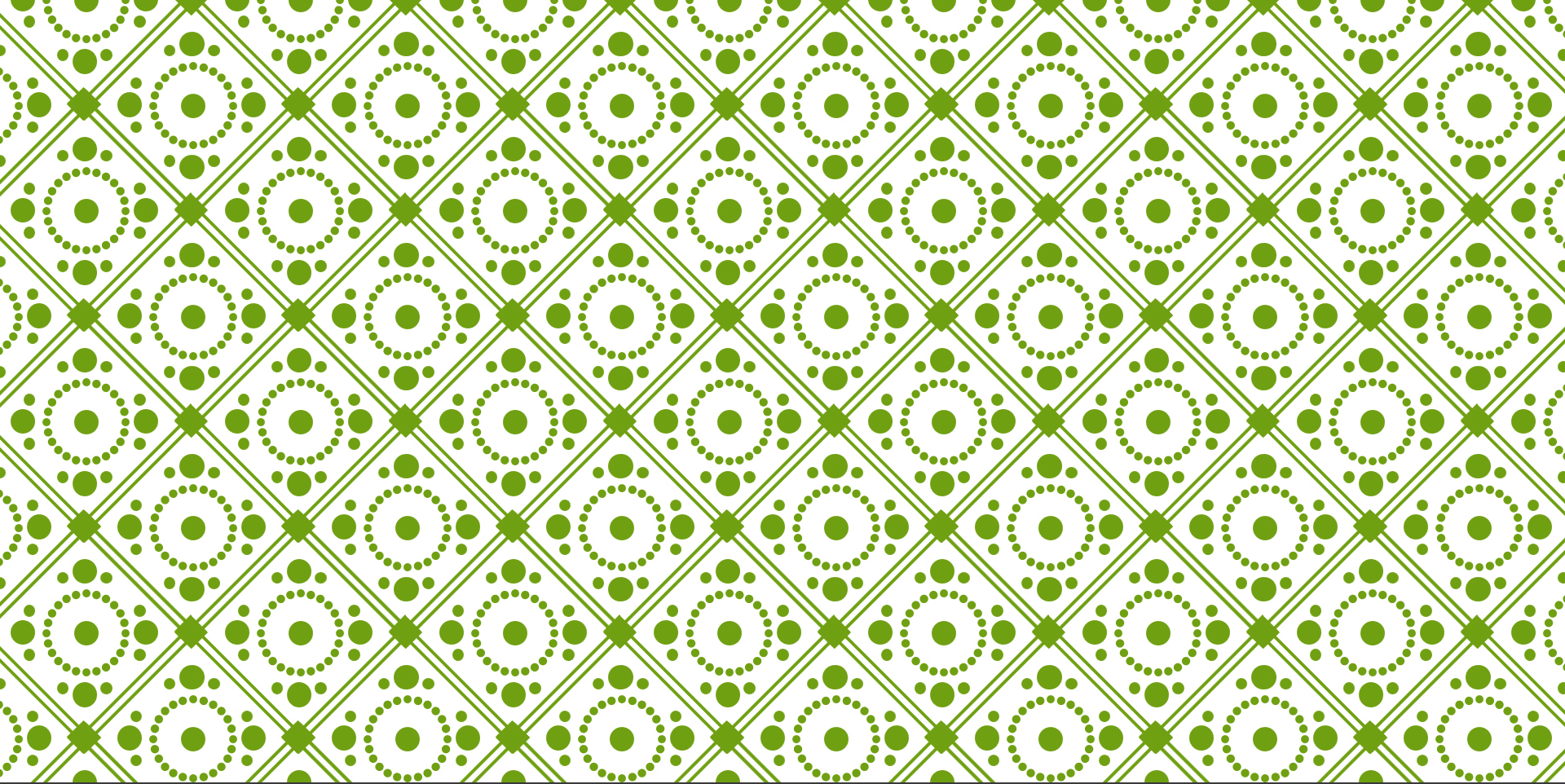
APLIKACE

Příklady analýz

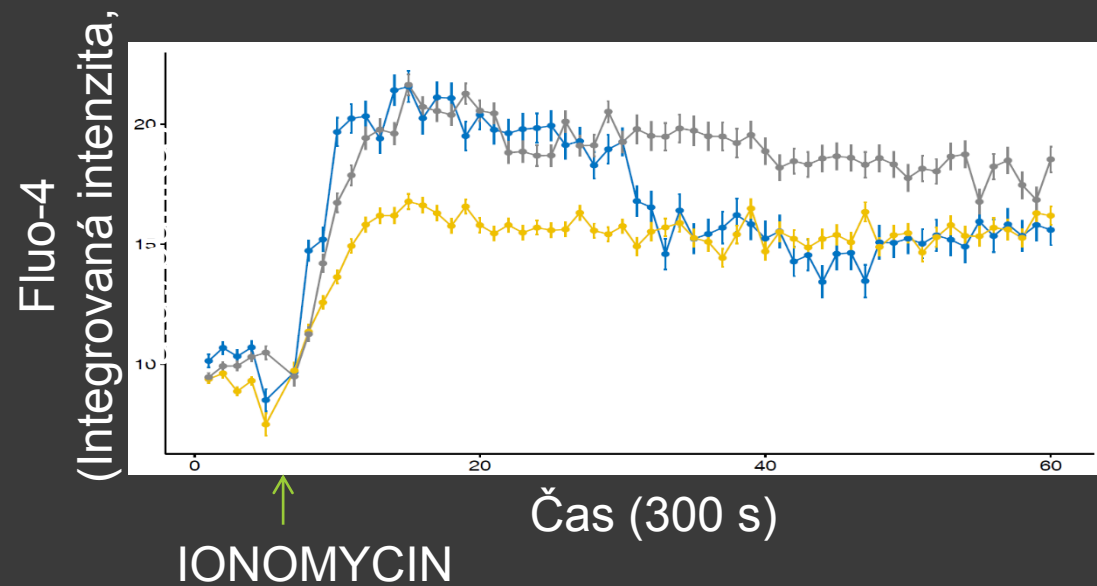
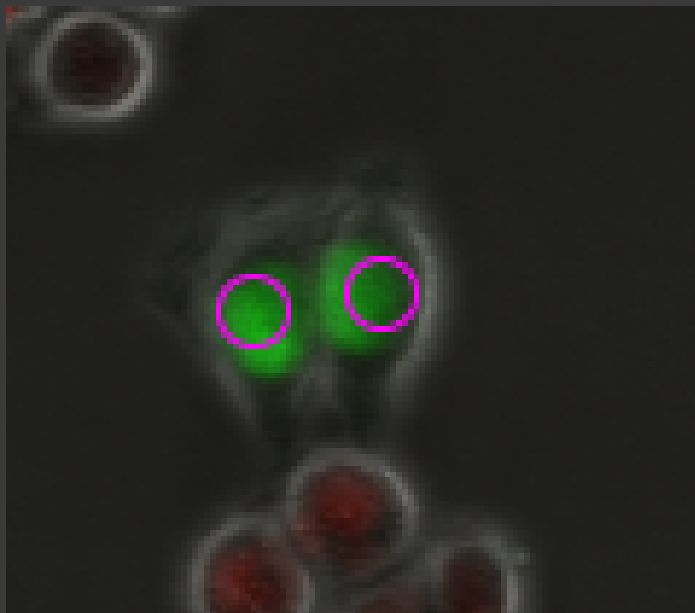
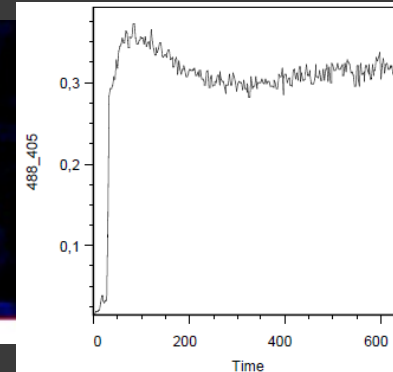
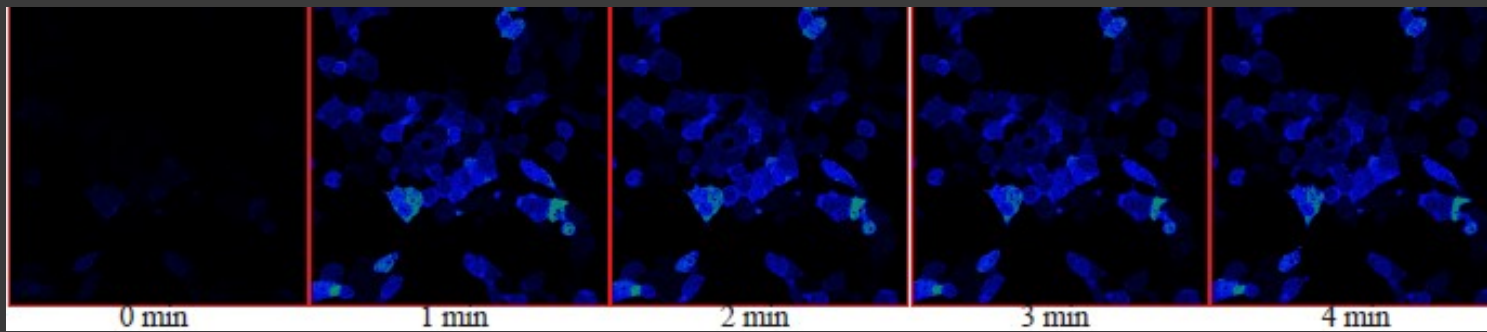
POČÍTÁNÍ BUNĚČNÝCH KOLONIÍ

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



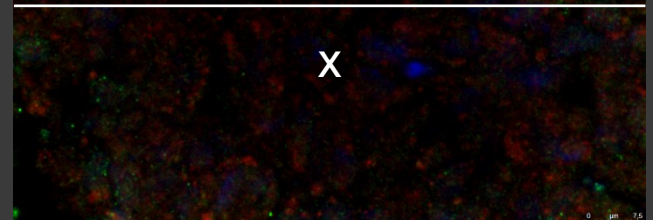
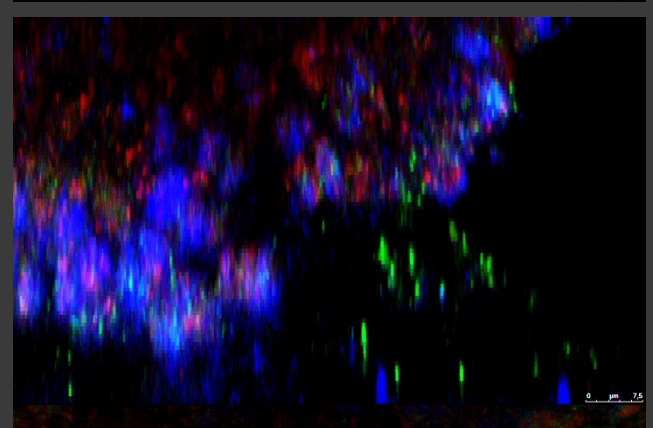
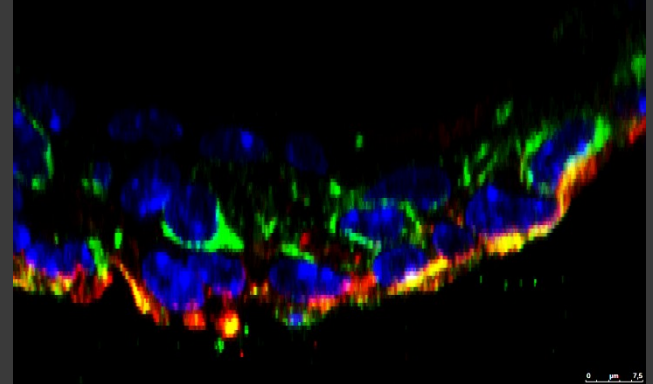
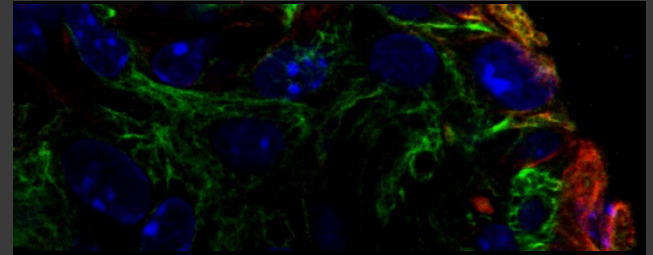
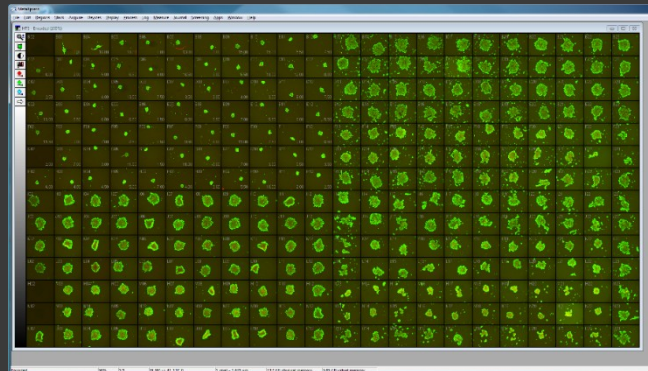
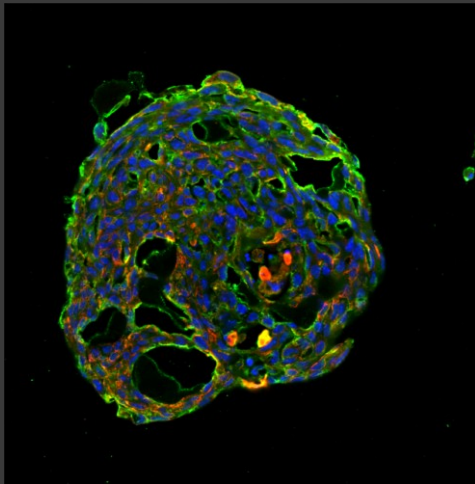
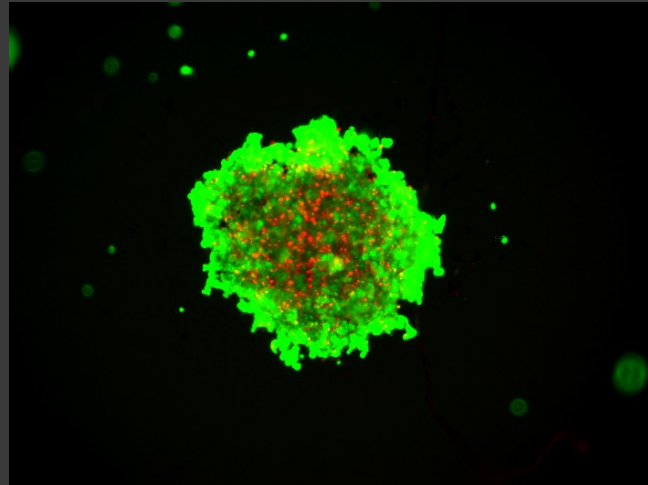
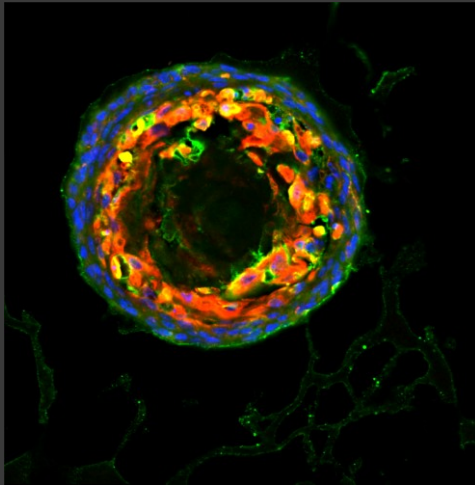


ANALÝZA ŽIVÝCH BUNĚK



3D ANALÝZY

- Kvantifikace objemu
- 3D projekce
- 3D kultivace



Z

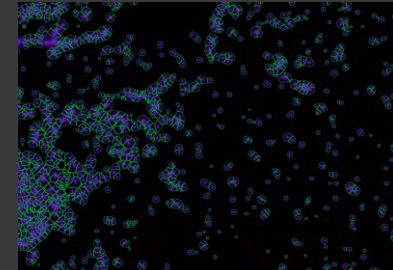
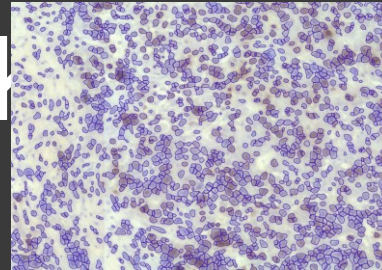
X

DETEKCE SFEROIDŮ A INVAZIVITY

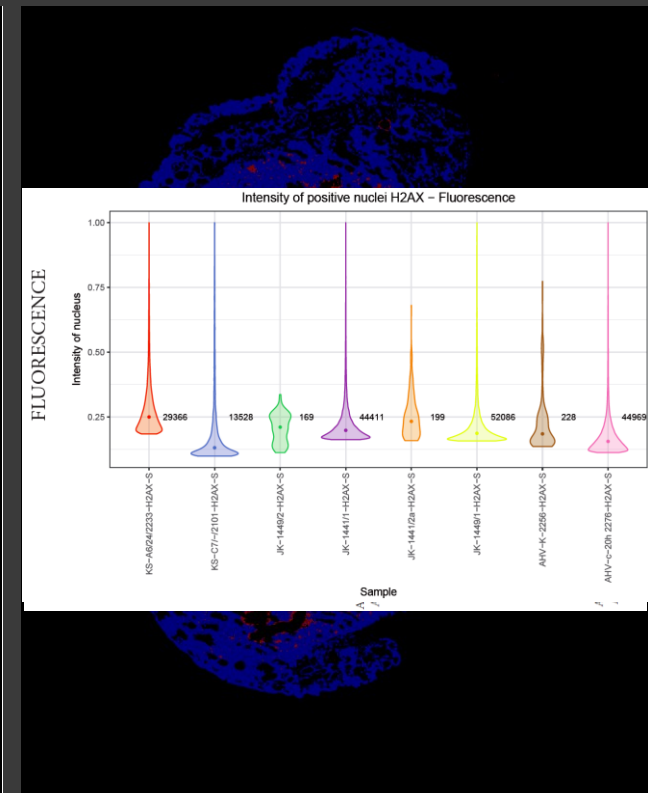
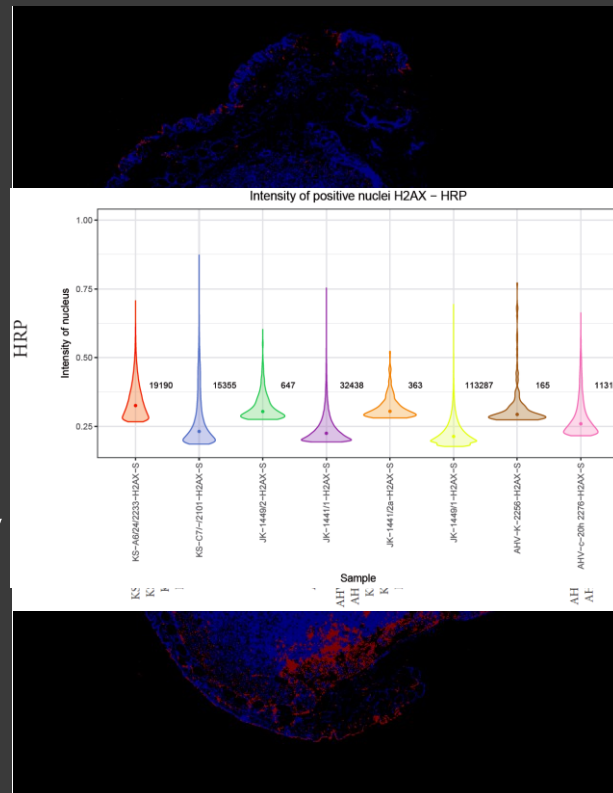
- Hightroughput
- Průchozí světlo
- Detekce sferoidů
- Detekce invadujících buněk
- Plocha



ANALÝZA TR



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



MORFOLOGIE – CELL PAINTING ANALÝZA

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

CELL PAINTING - POSTUP



Výsev buněk



Pátek

4 h

Speciální deska

Manuální,
4jamky/linie = 88
linií, 80% denzita

Značení



Pondělí

3 h

Zavedený
protokol

Poloautomatické

Snímání



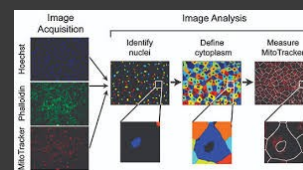
Pondělí

40 h

Zavedený
protokol

40x objektiv,
Adaptivní – 1tis.
buněk/jamka,
120 GB

Analýza obrazů



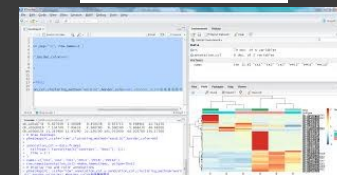
Čtvrtek

7 dní

3 protokoly

Klastr, Kvalita
obrazu, 1785
parametrů

Analýza dat



Další čtvrtek

2 h

Skript

Poloautomatické,
heatmapa, PCA, Vi-
SNE, Odlišné
parametry

CELL PAINTING - OBRAZKY

DAPI

Cy3

GFP

TxRed

Cy5

Hoechst 33342

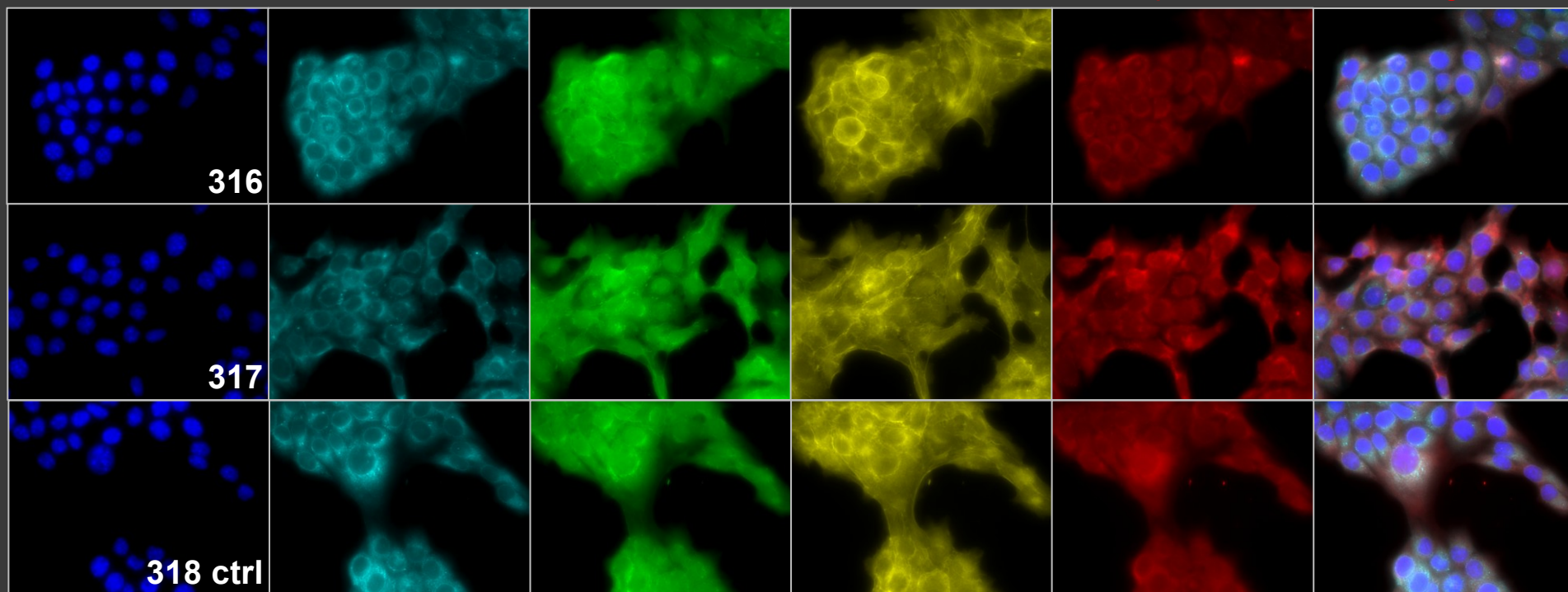
Alexa Fluor 488

Green

AF568, AF555

Deep Red

Merge



DNA

Concanavalin A
Glycolipids

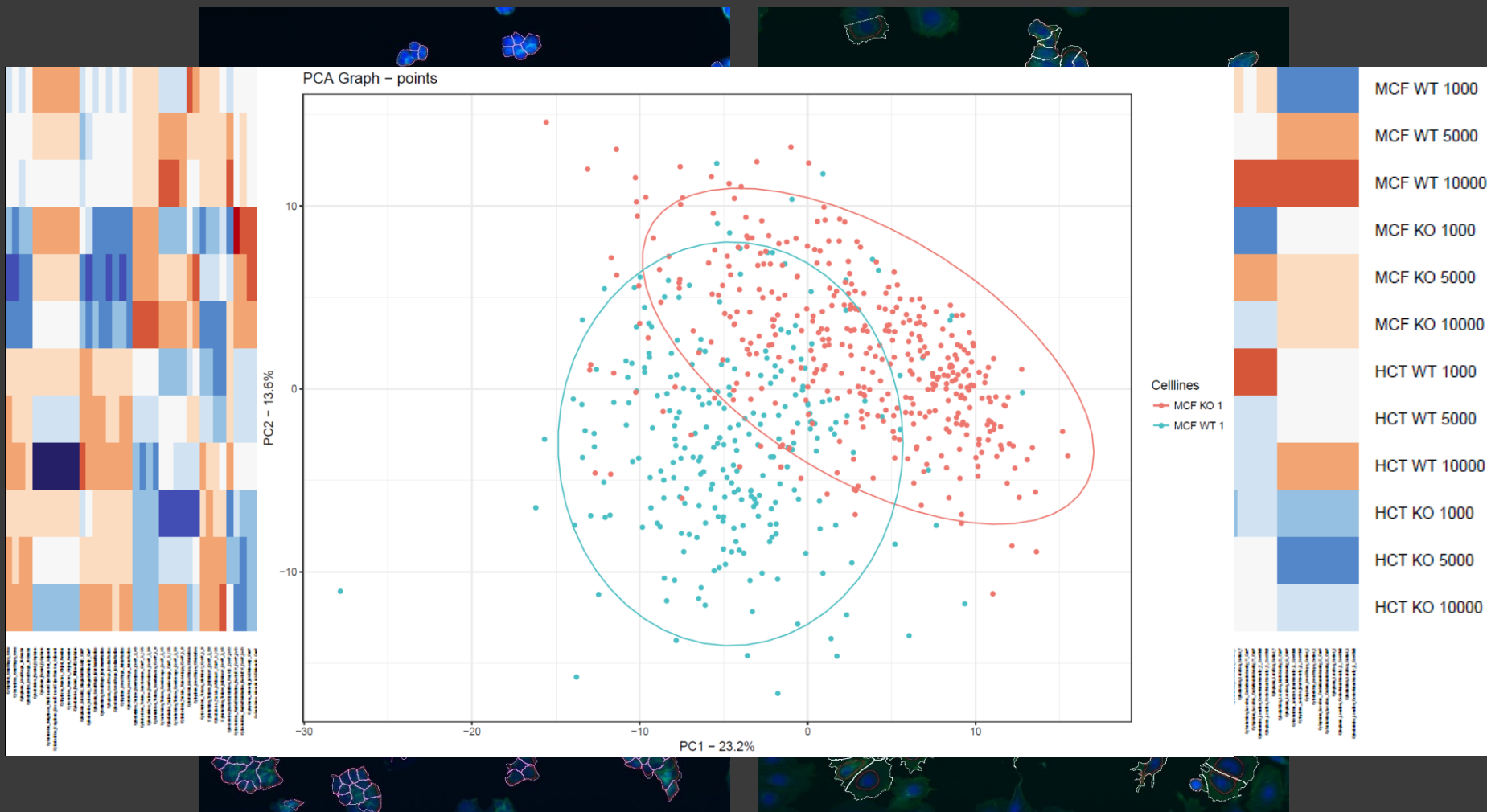
SYTO 14

Phalloidin
Wheat germ
agglutinin

MitoTracker

Hoechst,
Concanavalin A,
MitoTracker

CELL PAINTING - VÝSLEDKY

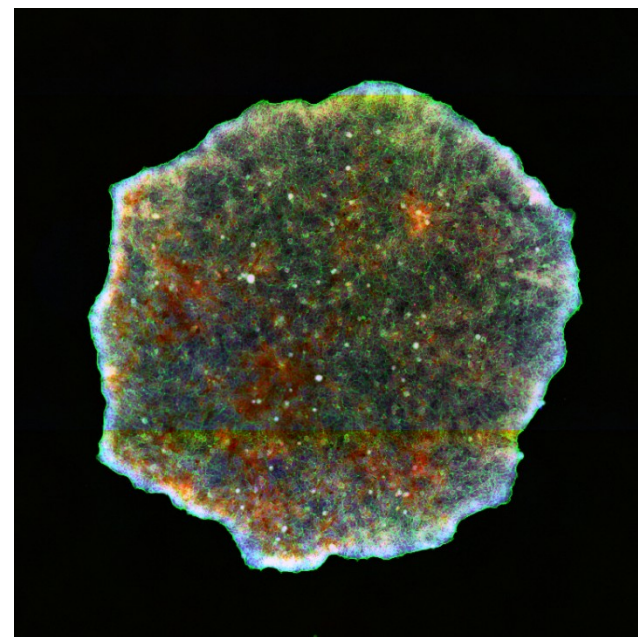


K ČEMU JE TO DOBRÉ?





MUNI



DĚKUJI ZA
POZORNOST

27.11.2023
Radek Fedr