



ZÁKLADNÍ PRINCIPY ANALÝZY OBRAZU

Příklady analýzy
obrazu

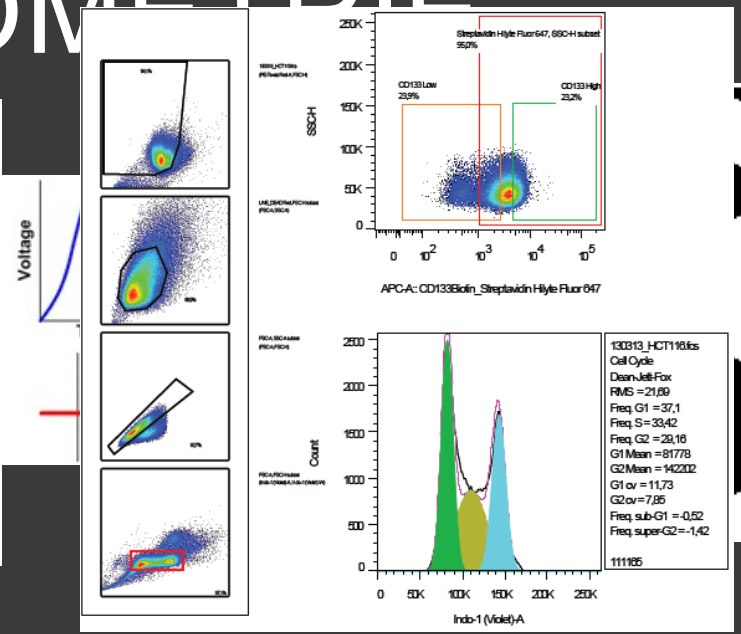
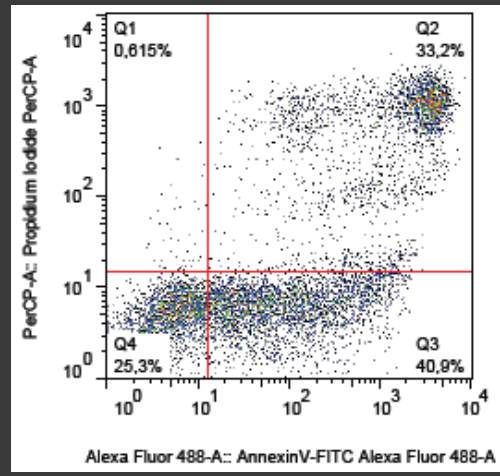
Radek Fedr

K ČEMU JE TO DOBRÉ?



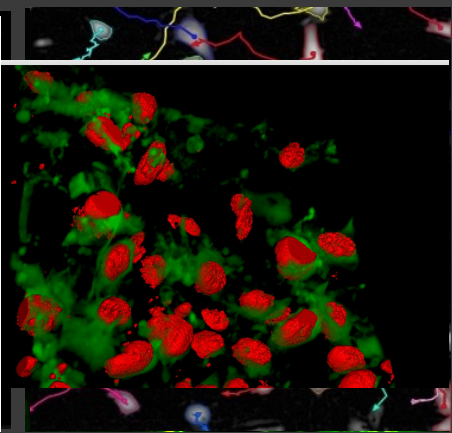
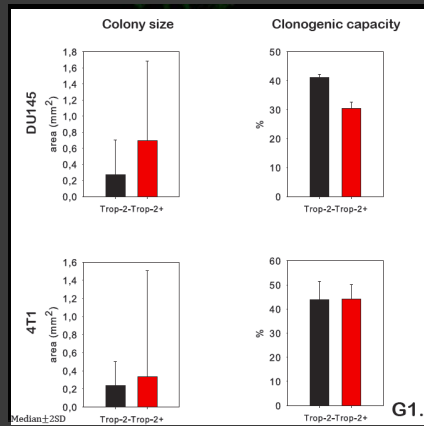
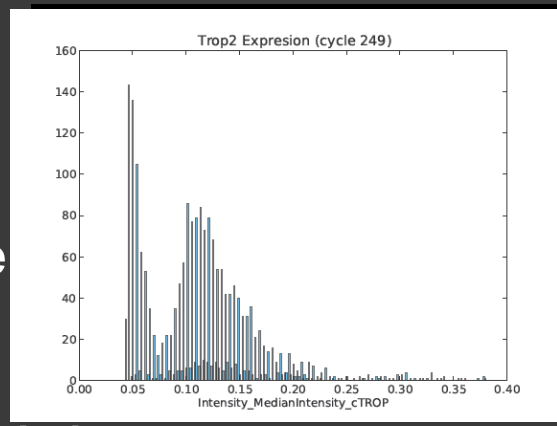
PRŮTOKOVÁ CYTOMETRIE

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



OBRAZOVÁ CYTOMETRIE

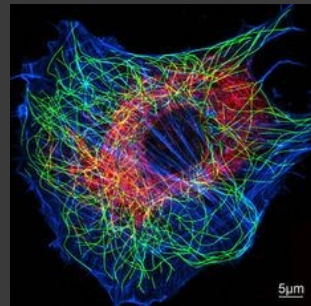
- Pixel
- Intenzita - pixel
- Distribuce
- Obraz, Projekce
- Graf, Model
- Tracking/Sledování



SROVNÁNÍ

Mikroskopie

Ukázka



Vzorek

2D, 3D

Rozlišení

100nm

Fluorescenční parametry

1-5

Rychlost

100 buněk/s

Data

100GB

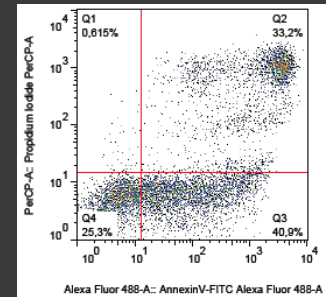
Kvantifikace

Přesná

Počet buněk

10^1 - 10^6

Průtoková cytometrie



Suspenze

1částice/1µm

10+

10000 buněk/s

100MB

Přesná

10^4 - 10^7

POSTUP

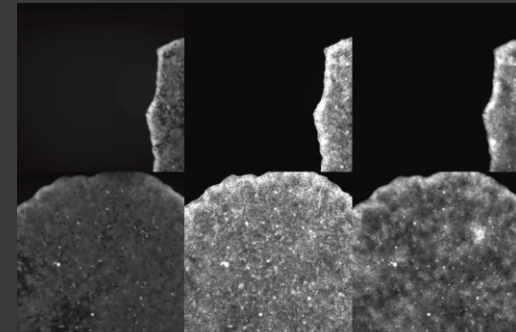
Příprava vzorku



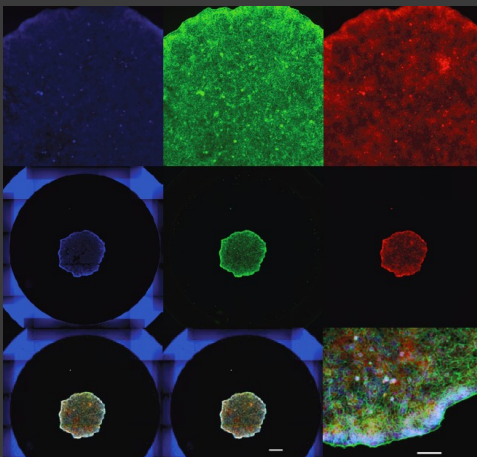
Snímání



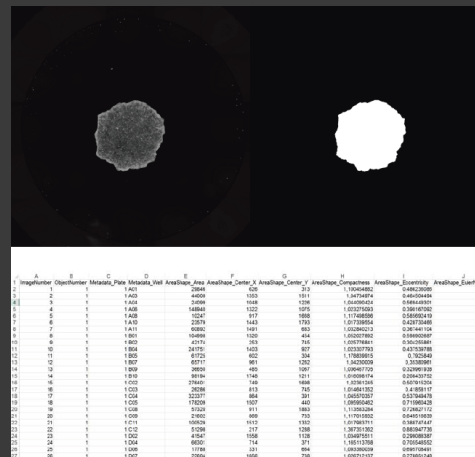
Uložení dat



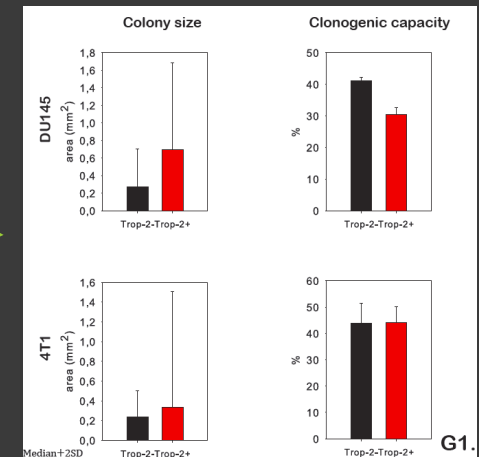
Zpracování



Analýza



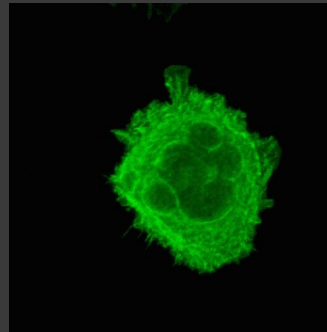
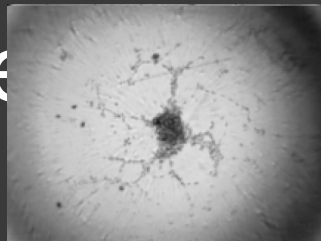
Vyhodnocení dat



PŘÍPRAVA A SNÍMÁNÍ

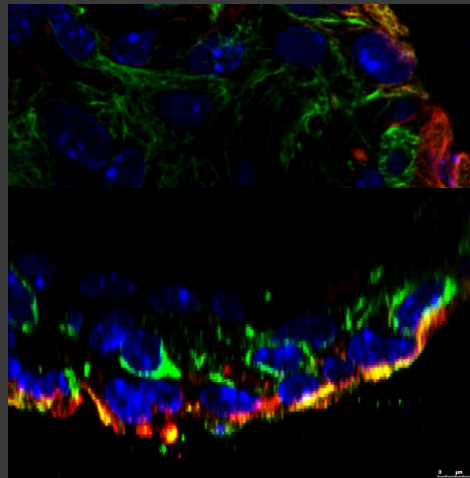
Experiment

- živé
- fixované
- 2D
- 3D



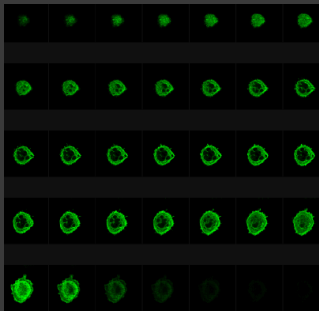
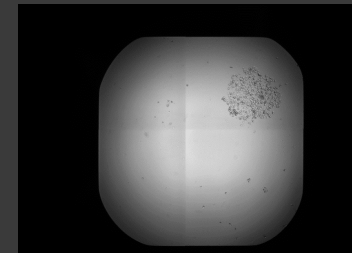
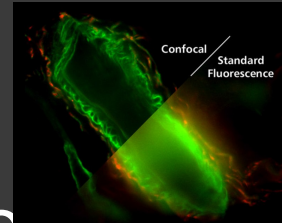
Vzorek

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

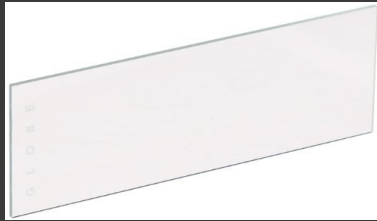


Mikroskopie

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

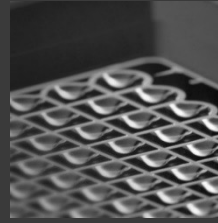


PŘÍPRAVA A SNÍM



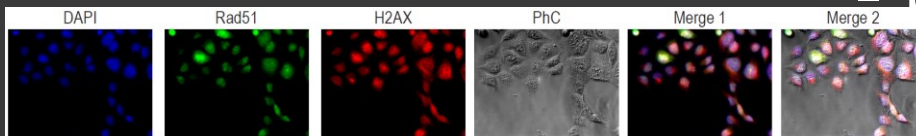
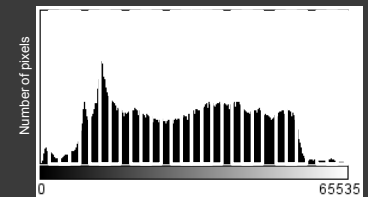
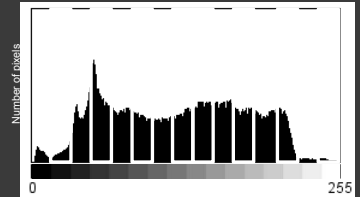
Plast

- podložní sklo
- ztenčené dno
- více-jamkové desky

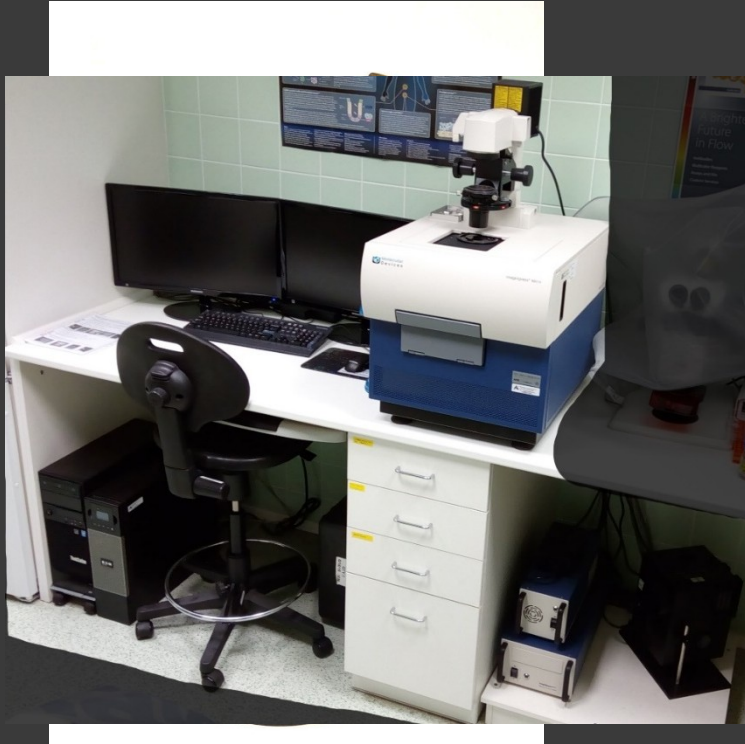


Rozlišení

- objektiv
- bitová hloubka
- počet barev



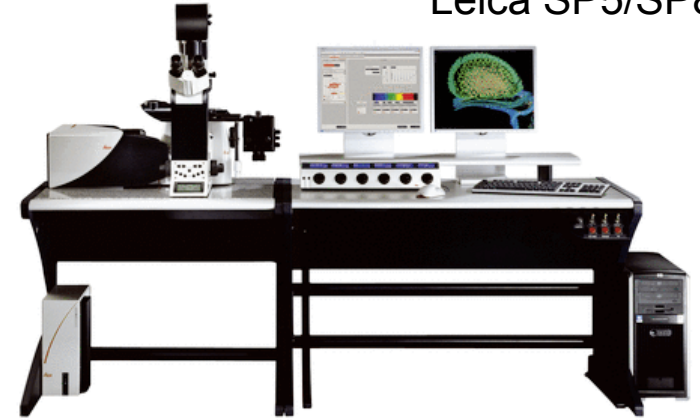
SNÍMÁNÍ



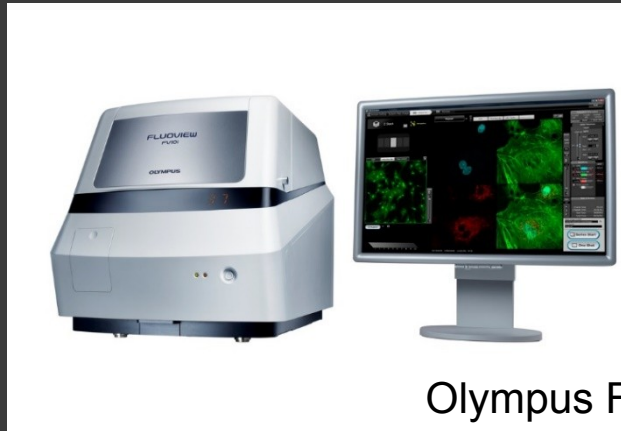
- Příprava
- Automatické
- Zpracování vzorku
- Popisné
- Xylohydrické

MIKROSKOP

Leica SP5/SP8



Olympus/Leica



Olympus FV10i



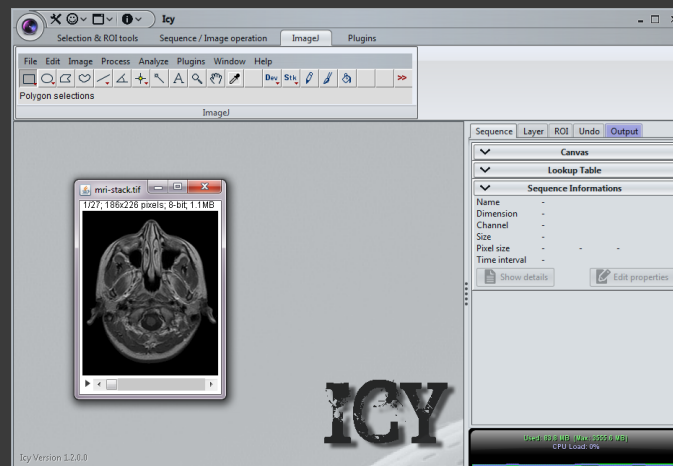
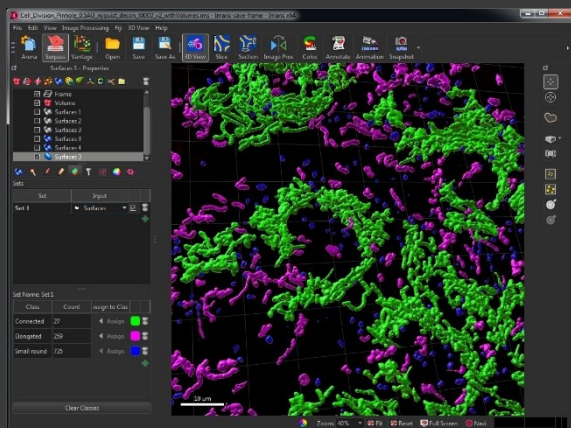
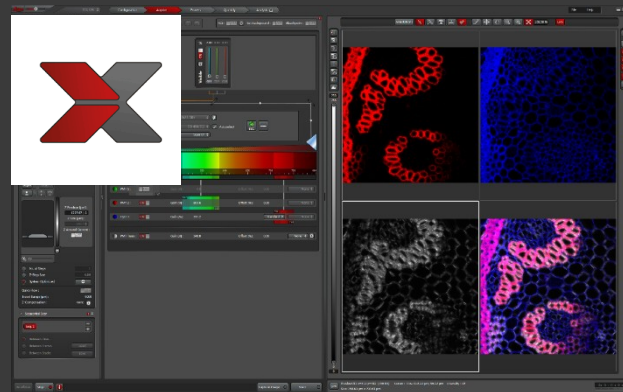
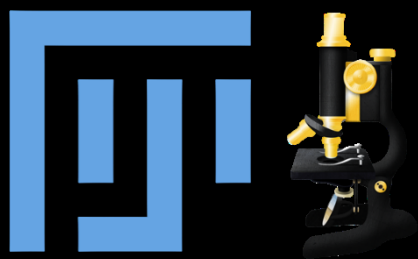
TissueFAXS Plus

Olympus IX70

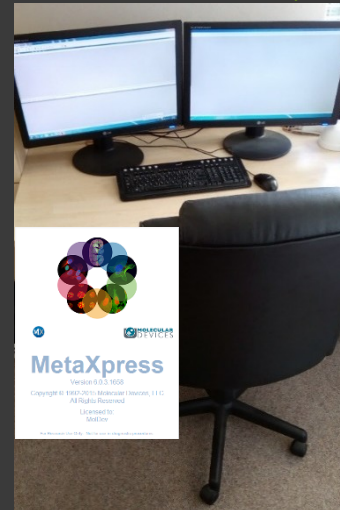


MD ImageXpress
Micro

SOFTWARE



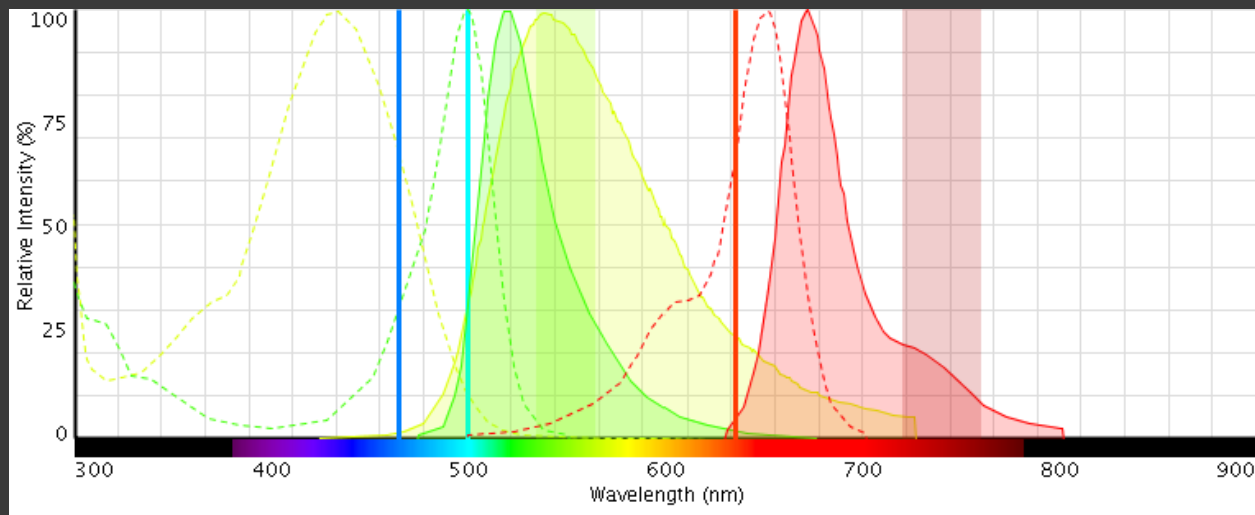
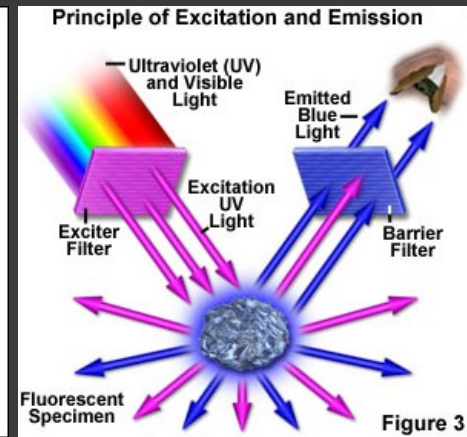
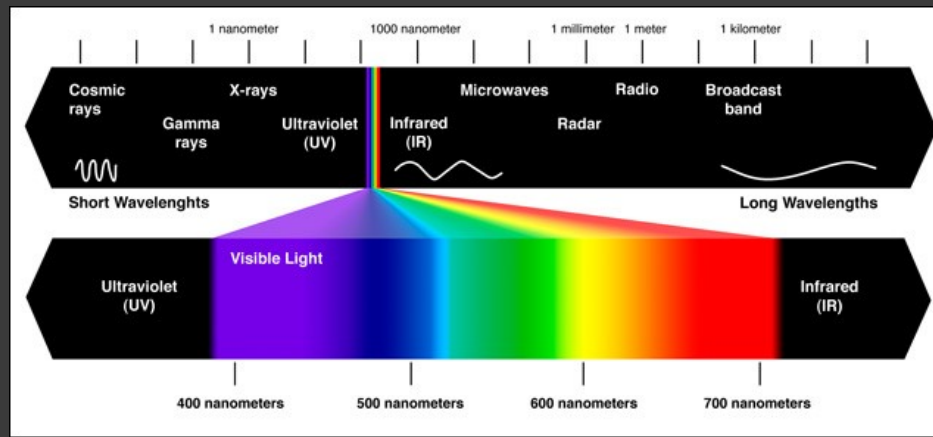
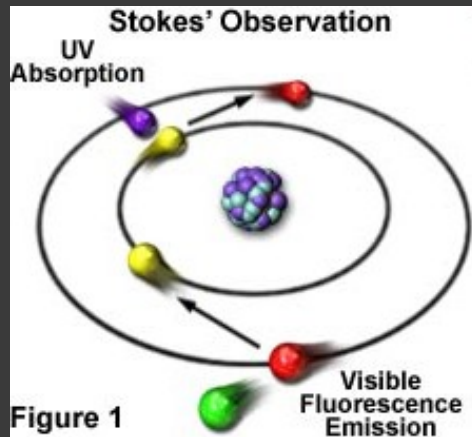
DATA



“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.”

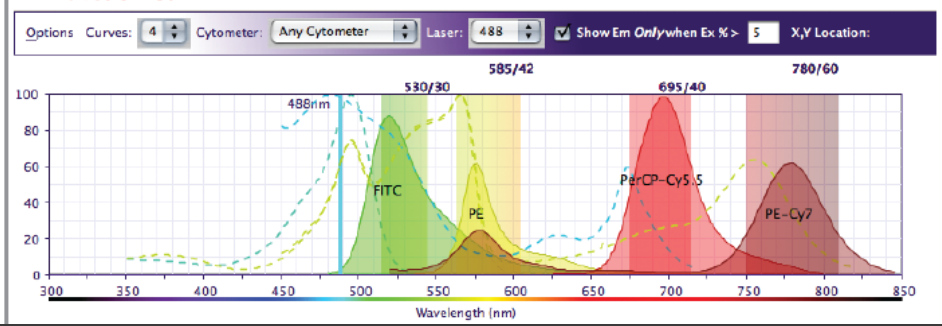
Bill Gates

FLUORESCENCE



FLUOROCHROM

BD Fluorescence Spectrum Viewer A Multicolor Tool



Fluorofinder® Resources Build a Panel Saved Panels My Account

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:	Alexa Fluor 405	Alexa Fluor 488	Alexa Fluor 647	APC	APC-Alexa 750
	APC-eFluor 780	APC-Vio770	BB 515	Brilliant Violet 421	Brilliant Violet 510
	DyLight 405LS	DyLight 488	ECD	eFluor 450	FITC
	PE	PE-Cy7	PE-Dazzle 594	PerCP	PerCP-Cy5.5
	PerCP-Vio700	PE-Vio770	VioBlue	VioGreen	PerCP-eFluor 710
	^ less				
CD44	Human	Mouse	IgG2a	Any	
+ primary/secondary					
Fluorochrome Availability:	Alexa Fluor 405	Alexa Fluor 488	Alexa Fluor 647	APC	APC-Alexa 750
	APC-eFluor 780	APC-Fire 750	APC-H7	APC-Vio770	APC-Cy7
	^ more				
Live/Dead	All Species	Any	Any	Any	
+ primary/secondary					
Fluorochrome Availability:	FVS450	FVS510	FVS520	FVS570	FVS620
	FVS700	FVS780	Ghost Dye Red 710	Ghost Dye Red 780	FVS660
	^ more				

■ Síla fluorescence fluorochromů není stejná!

■ spectraviewer

■ www.fluorofinder.com

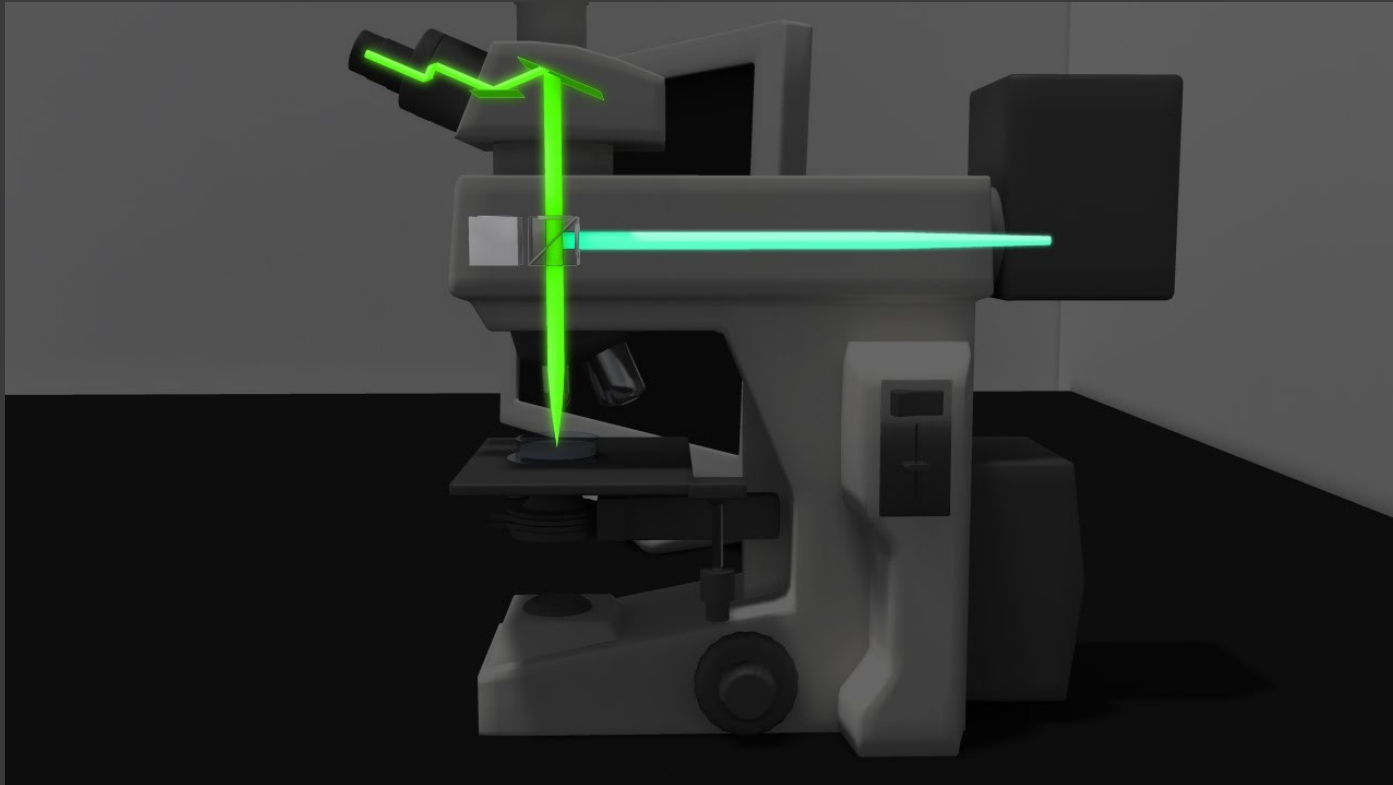
FLUOROCHROM

- 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.

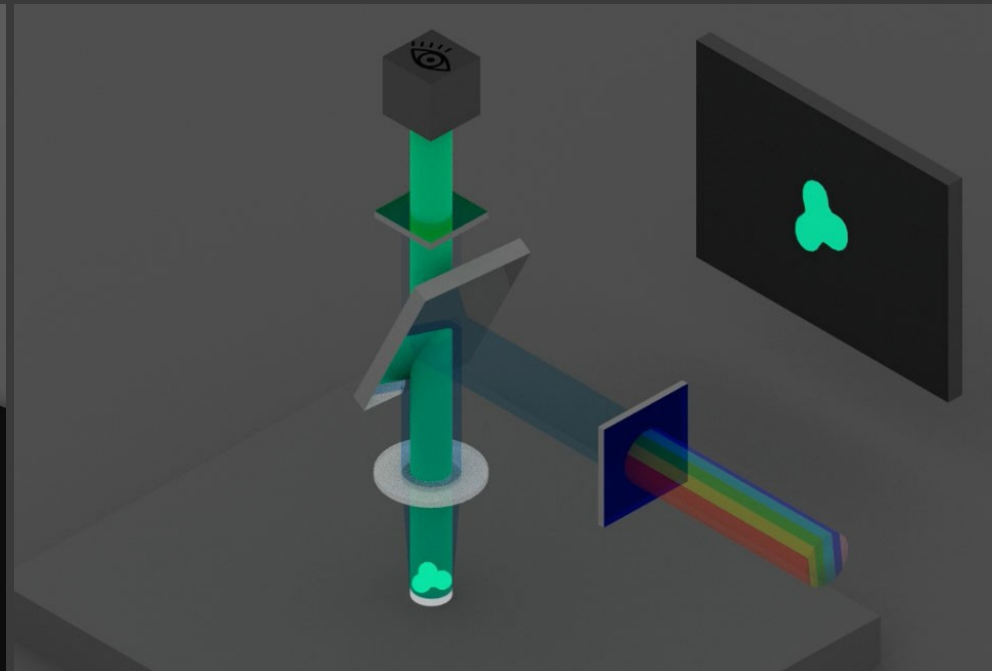
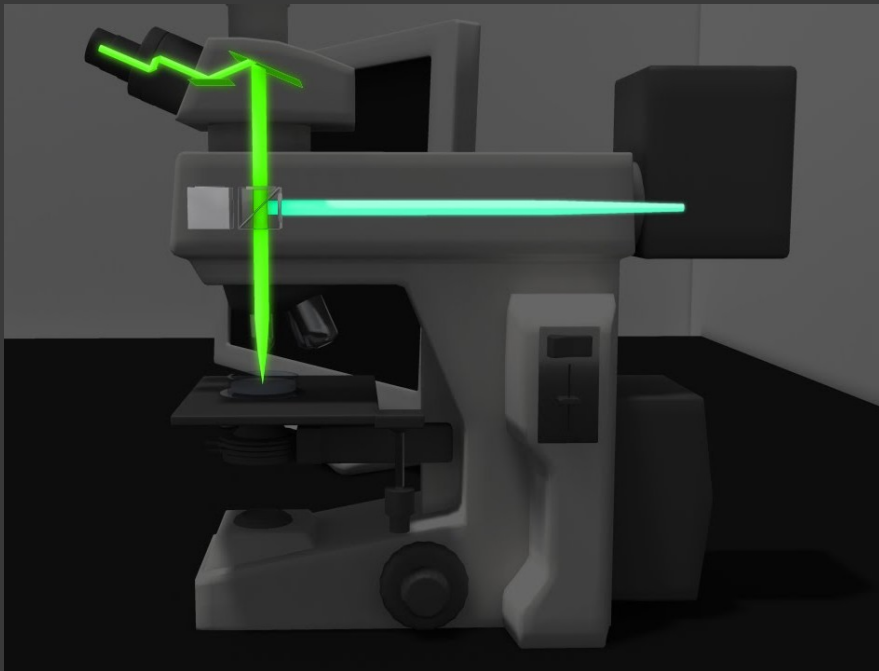
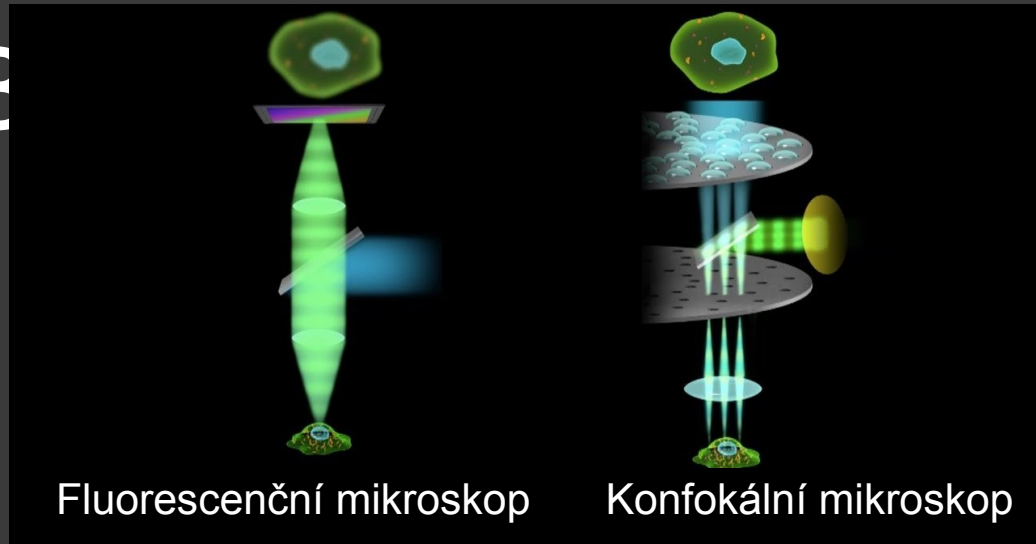
SNÍMÁNÍ OBRAZU

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

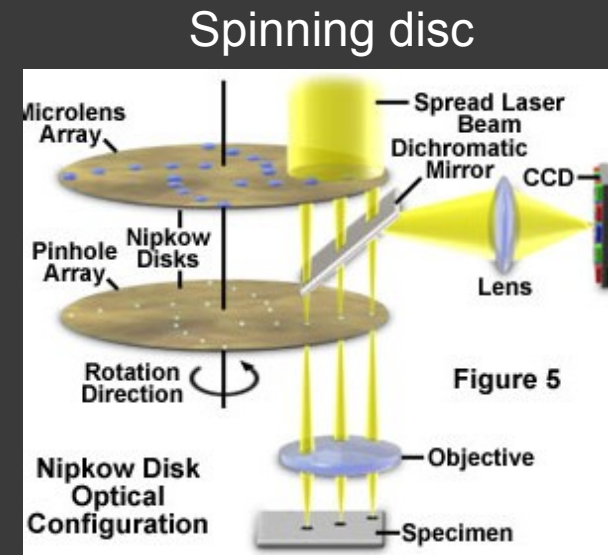
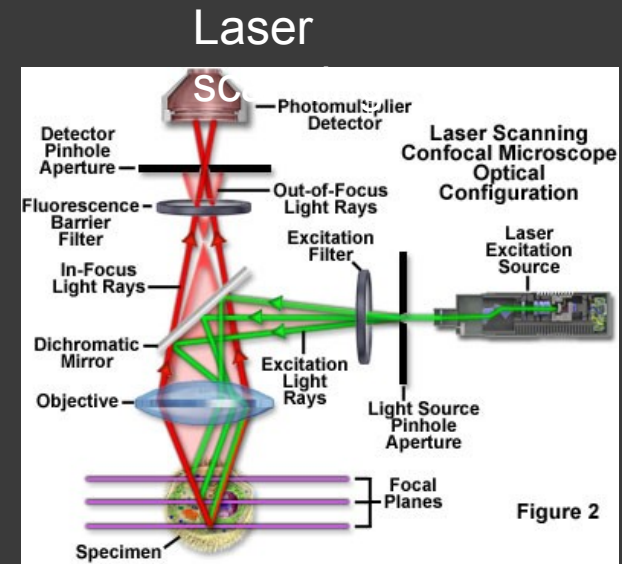
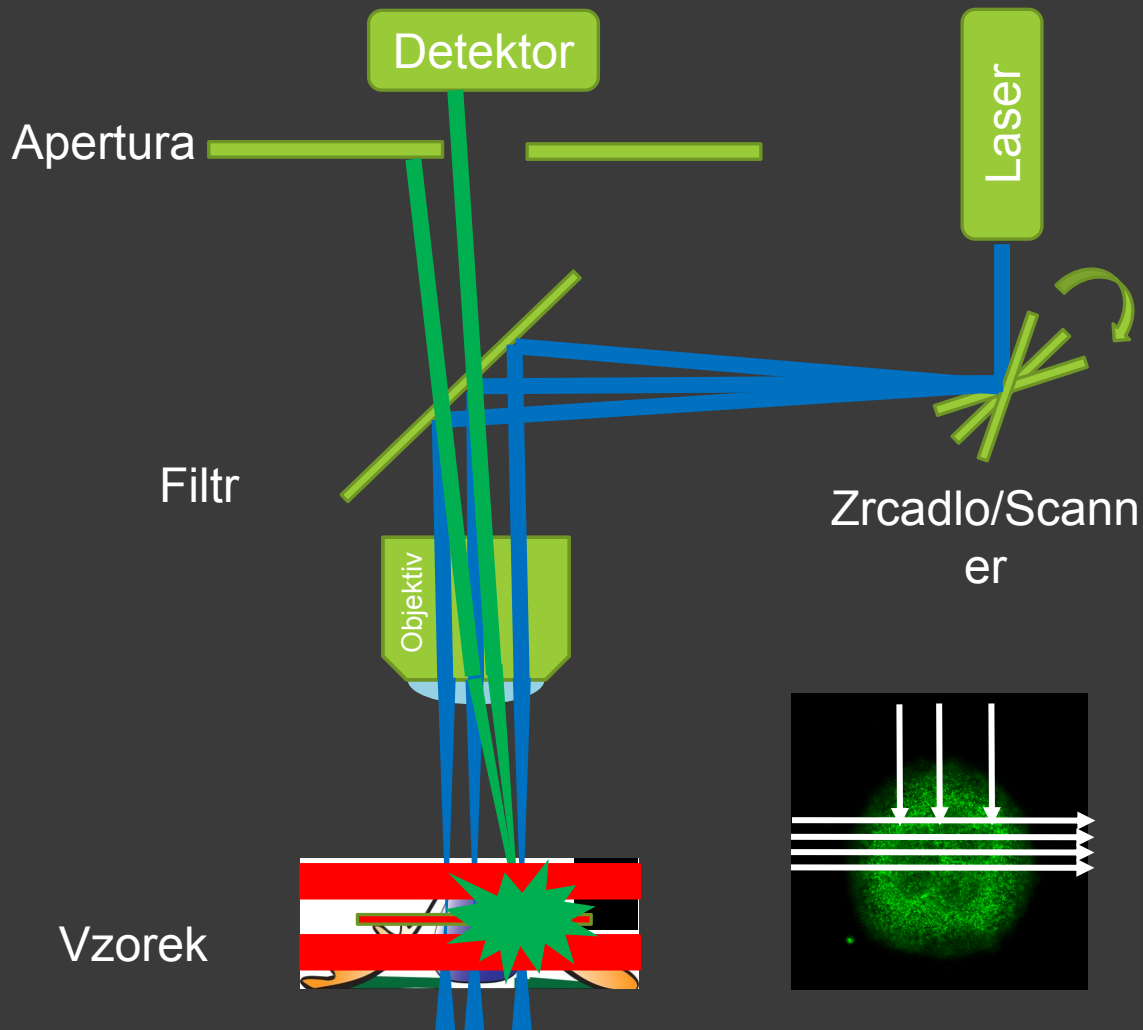


SNÍMÁNÍ OB

- Fluorescenční mikroskop
- vs.
- Konfokální mikroskop



SNÍMÁNÍ OBRAZU

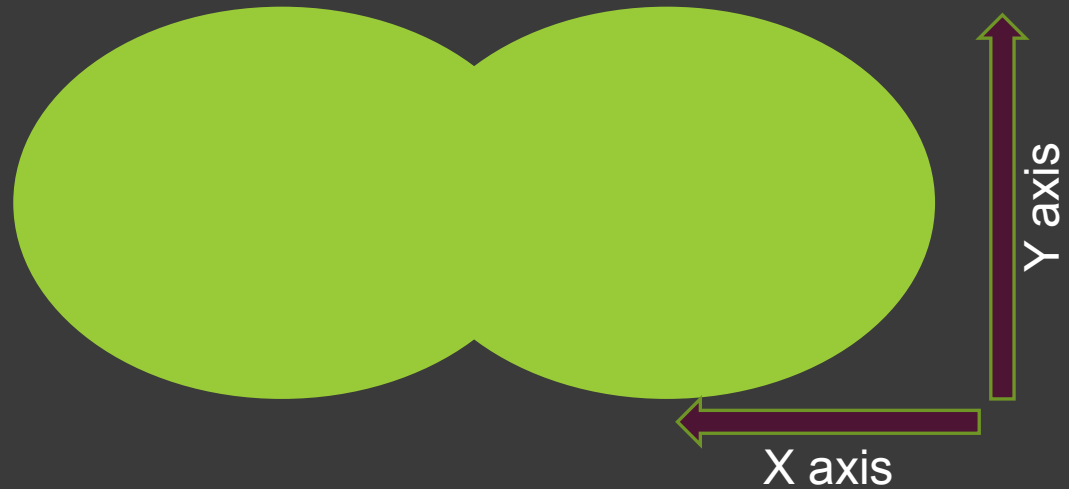
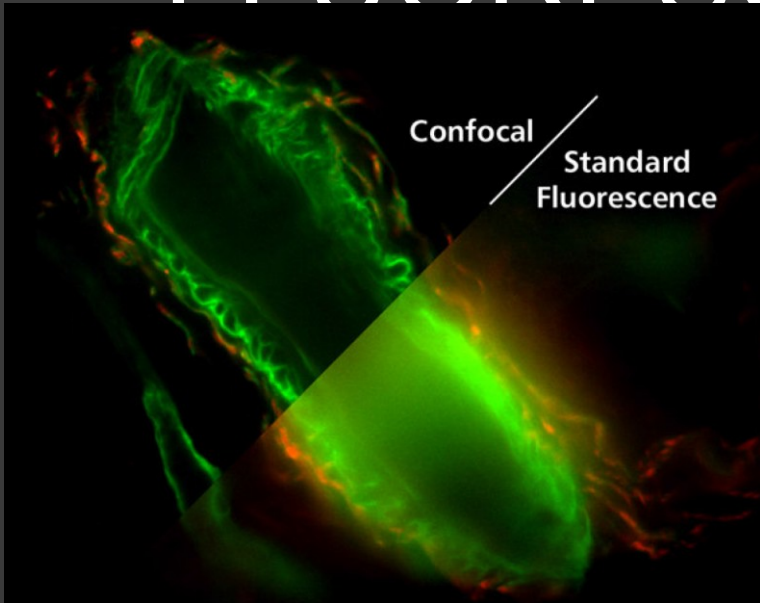


KONFOKÁLNÍ MIKROSKOP

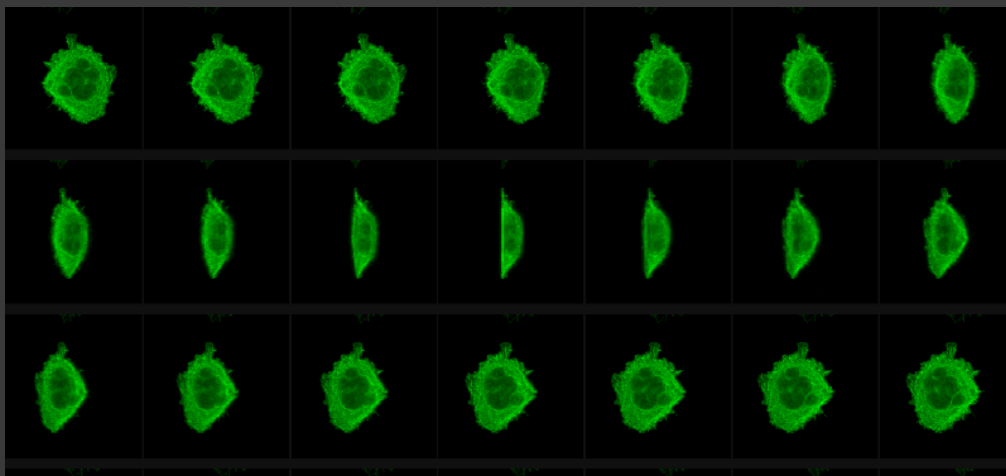
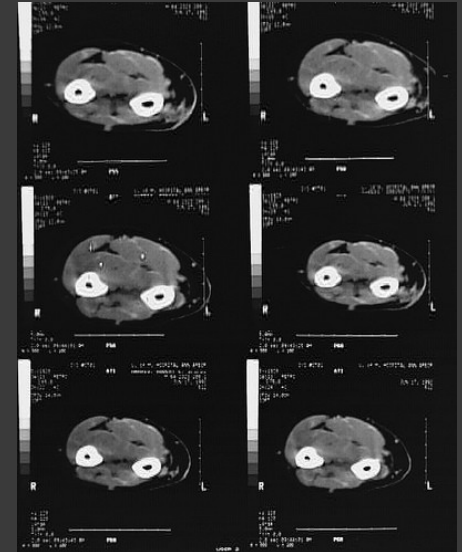
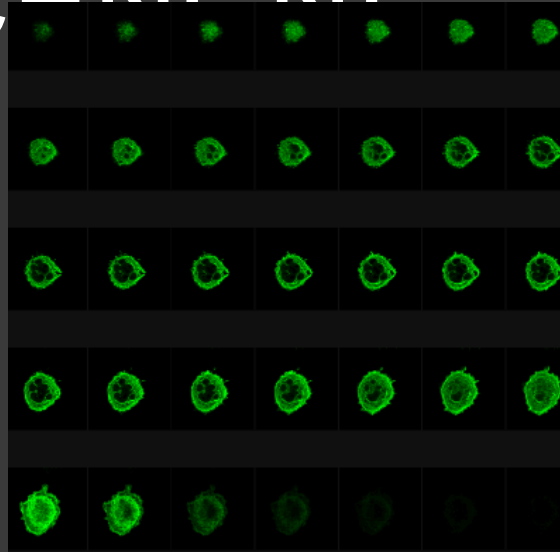
Leica



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)

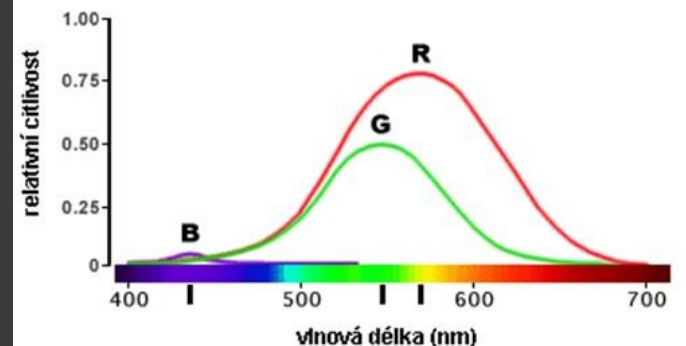
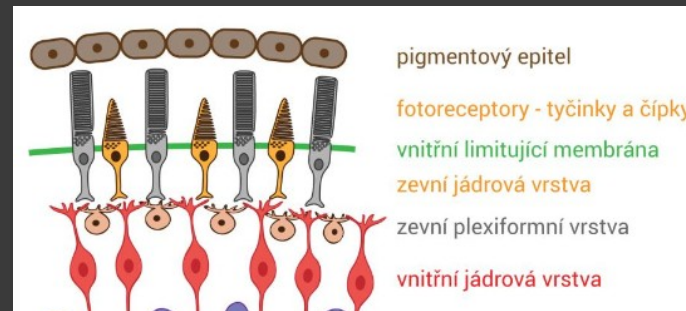
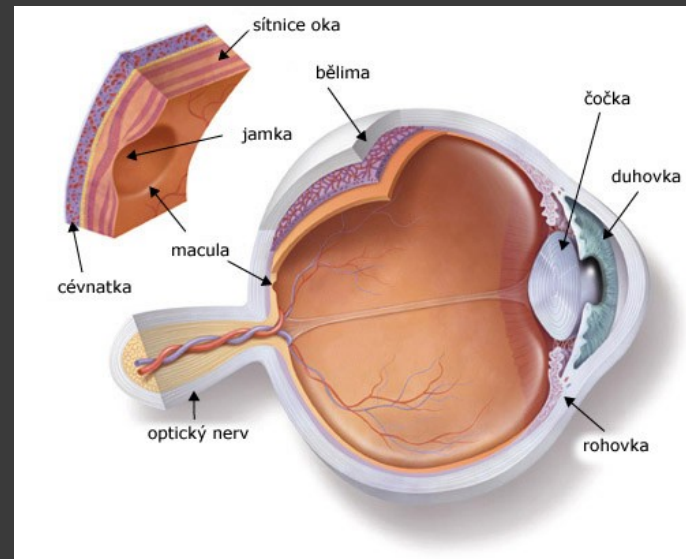
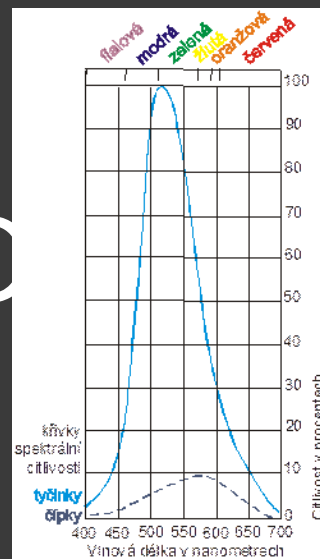
- 400-500nm Modrá – 4% 440 nm
- 500nm-600nm Zelená - 32% 540nm
- 600-700nm Červená – 64% 570 nm

- 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

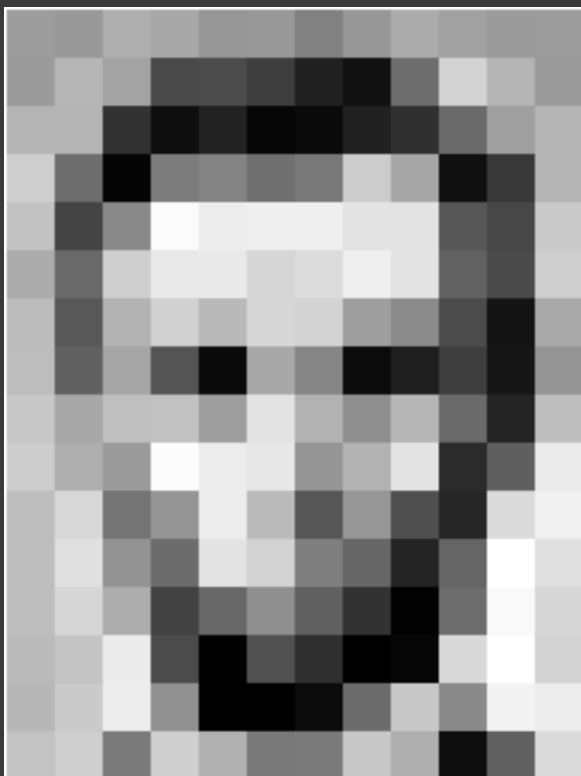


OBRAZ

2D tabulka pixelů

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

0 255

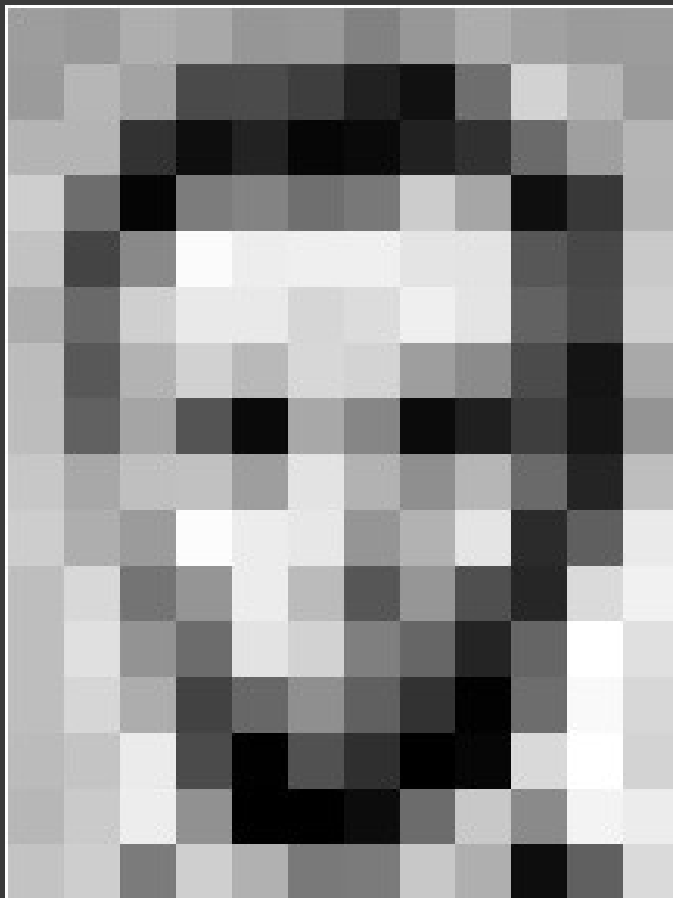
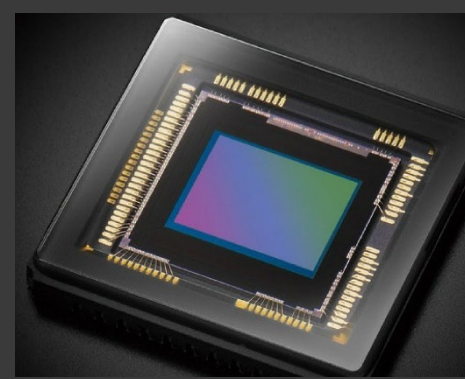


157	153	174	168	150	152	129	151	172	161	155	156
155	182	163	74	75	62	33	17	110	210	180	154
180	180	50	14	34	6	10	33	48	106	159	181
206	109	5	124	131	111	120	204	166	15	56	180
194	68	137	251	237	239	239	228	227	87	71	201
172	106	207	233	233	214	220	239	228	98	74	206
188	88	179	209	185	215	211	158	139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

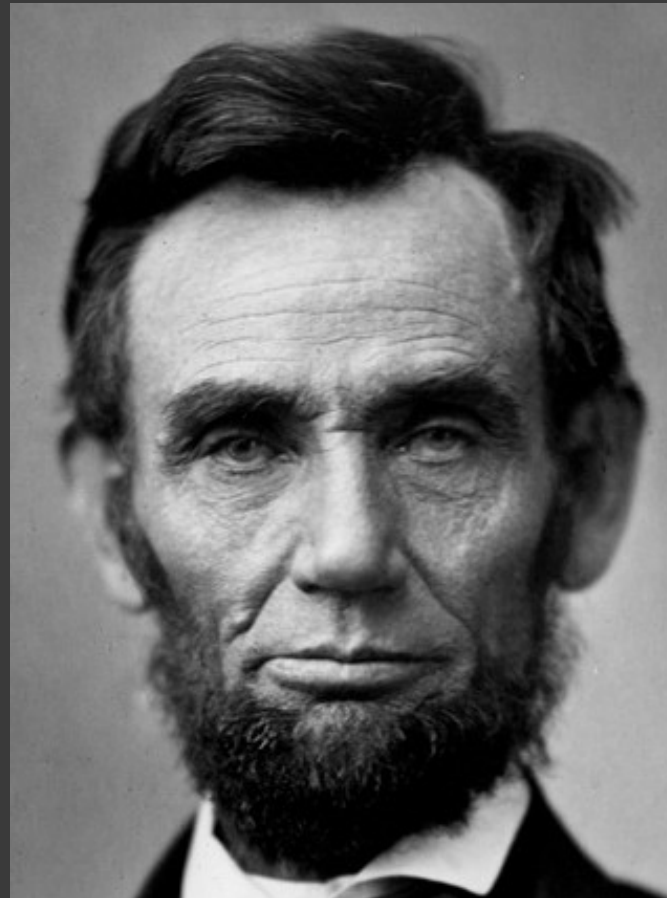
157	153	174	168	150	152	129	151	172	161	155	156
155	182	163	74	75	62	33	17	110	210	180	154
180	180	50	14	34	6	10	33	48	106	159	181
206	109	5	124	131	111	120	204	166	15	56	180
194	68	137	251	237	239	239	228	227	87	71	201
172	106	207	233	233	214	220	239	228	98	74	206
188	88	179	209	185	215	211	158	139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

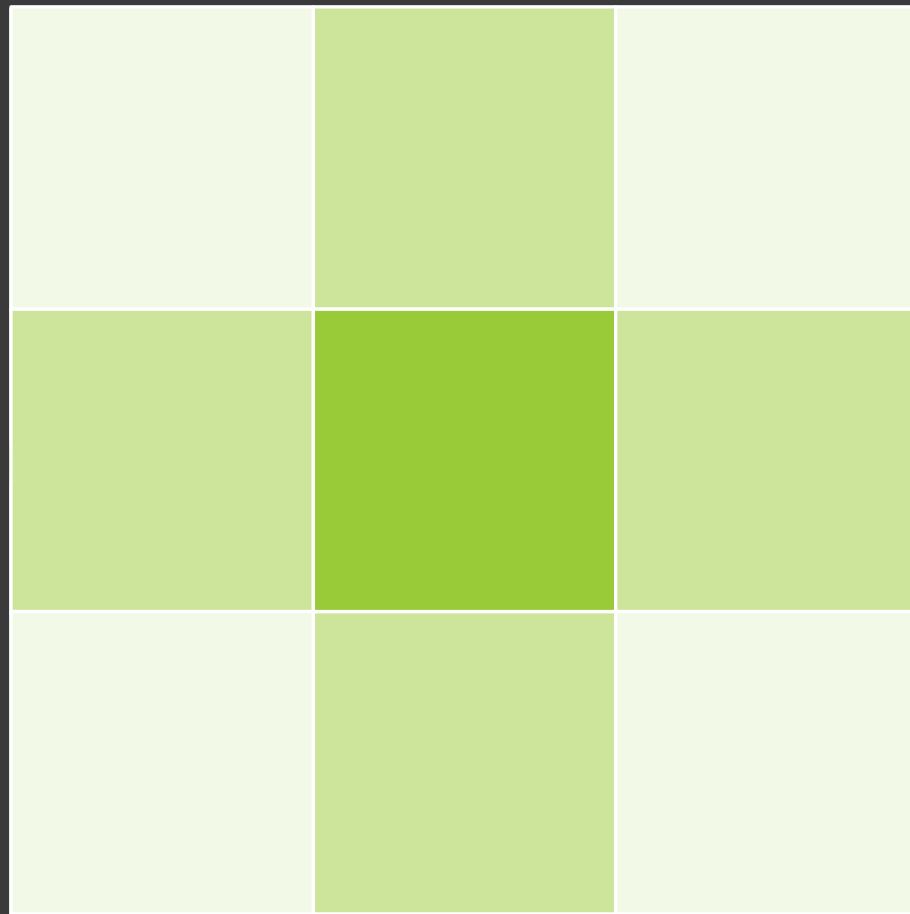
- počet pixelů
- CMOS/CCD čipy
- dpi – dots per inch
- tvar objektu vs. px
- zvětšení objektivu
- rozlišení objektivu



ROZLIŠENÍ



1 x 1 px



3 x 3 px

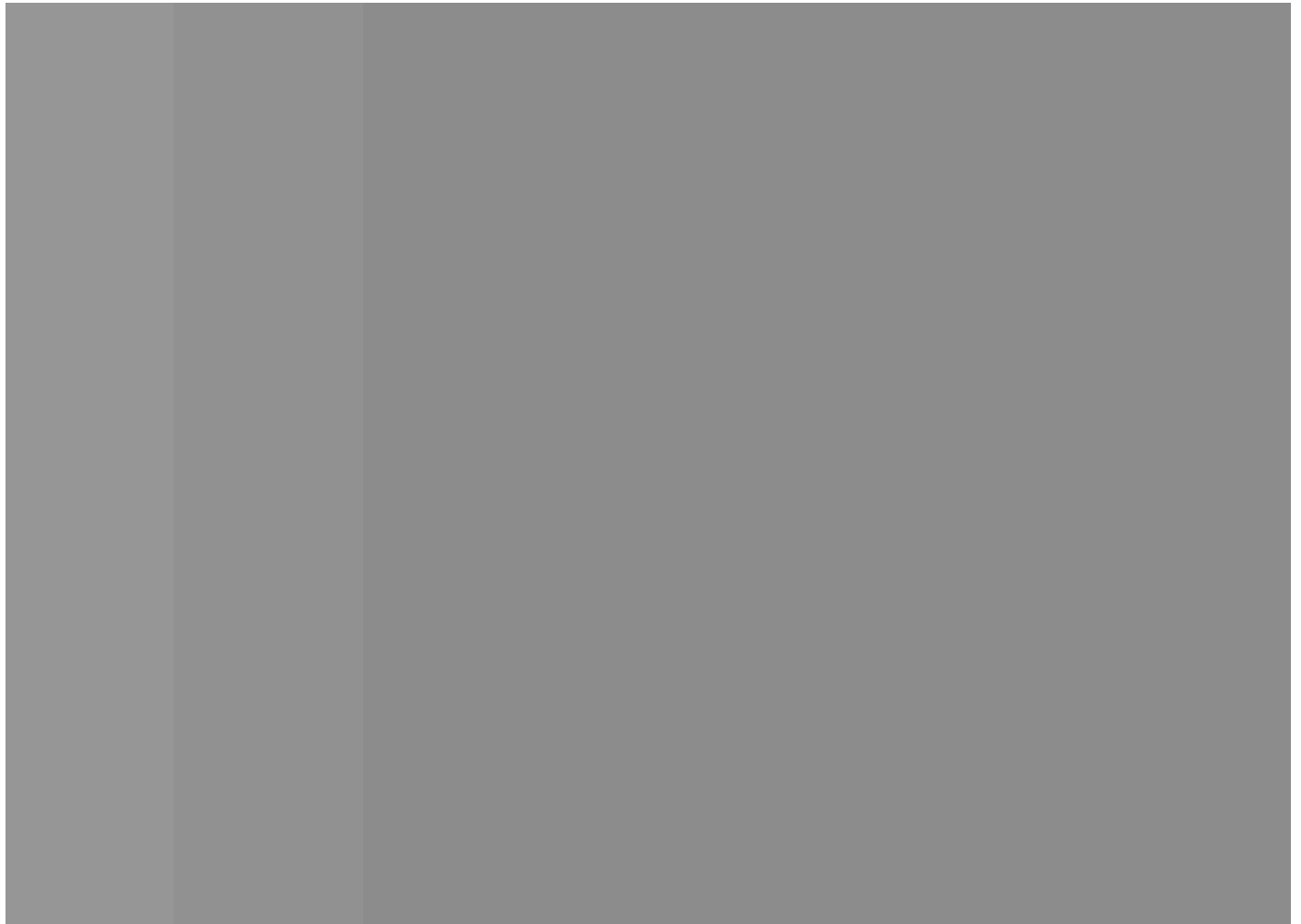
STUPNĚ ŠEDI

- Hladiny 150, 145, 140
- 30 stupňů šedi



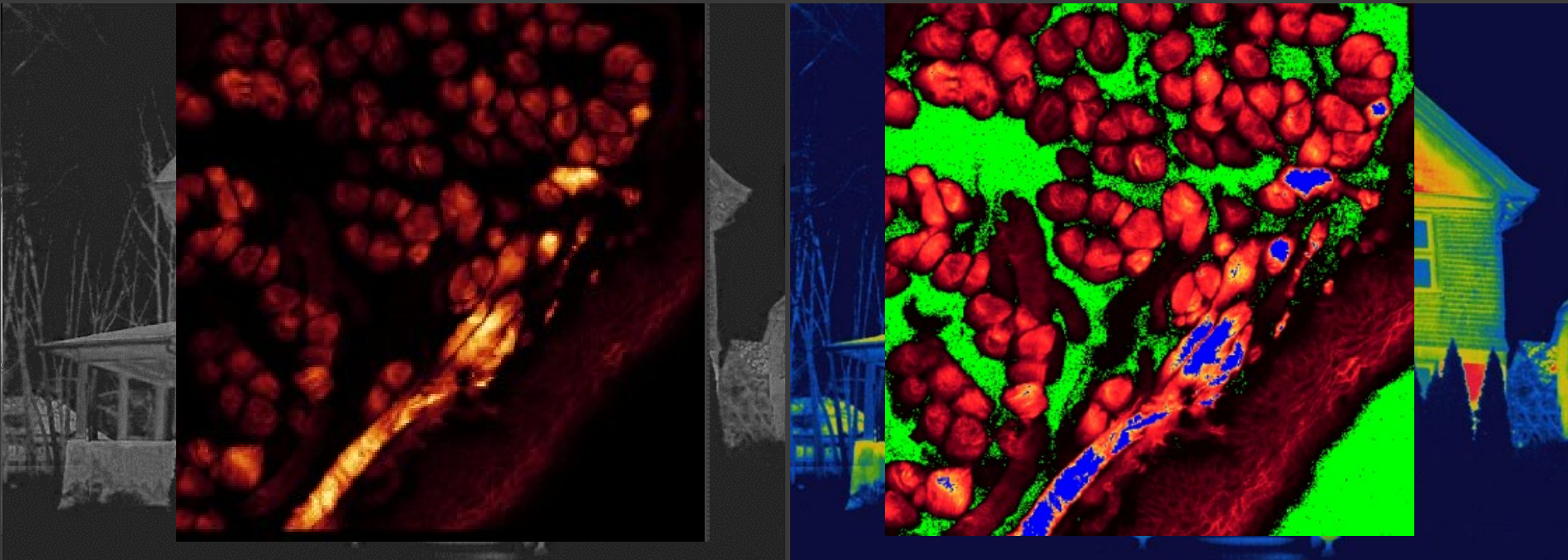
- okolo 900 stupňů šedi relativně

STUPNĚ ŠEDI



BAREVNÉ KÓDOVÁNÍ

- 10 milionů barev



ImageJ:
Analyze →
Histogram
Image → Type

HISTOGRAM – BITOVÁ HLOUBKA

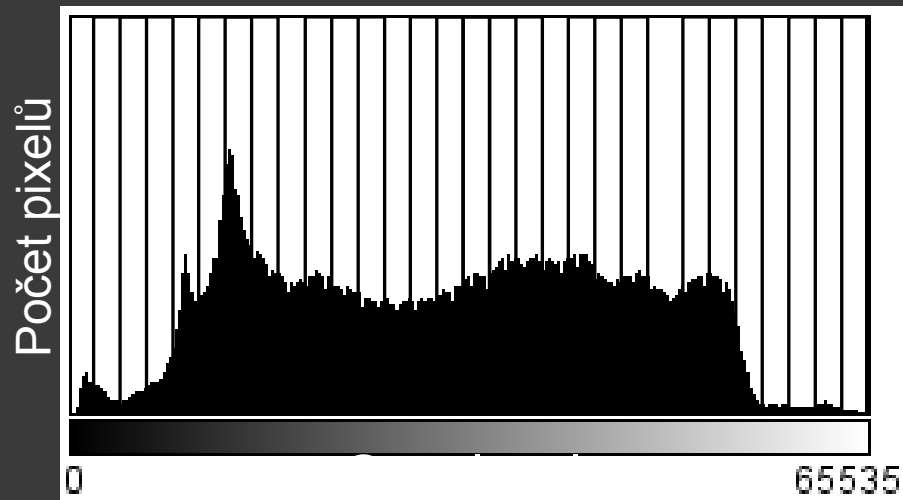
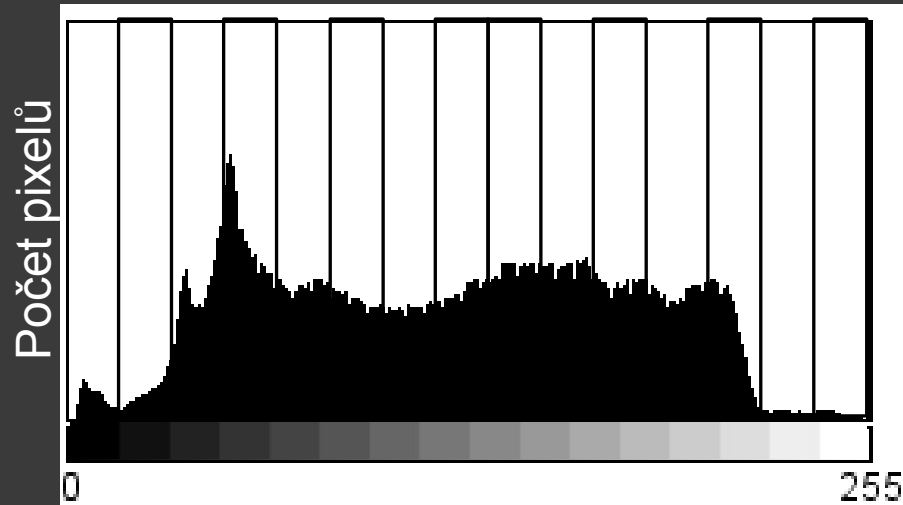
Originální obrázek

Histogram

Bitová
hloubka

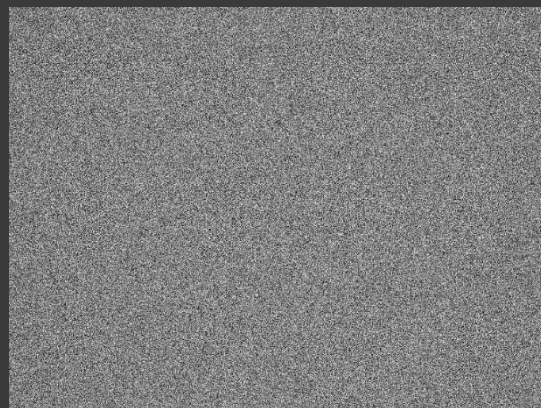
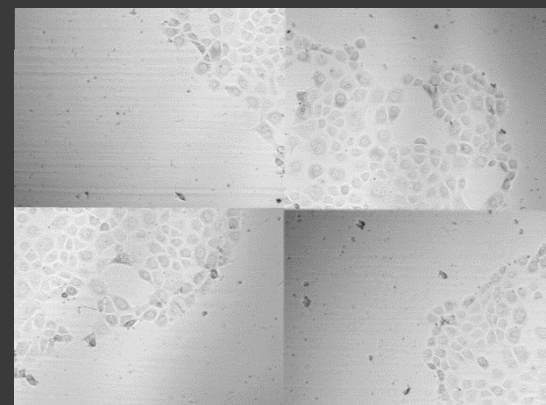
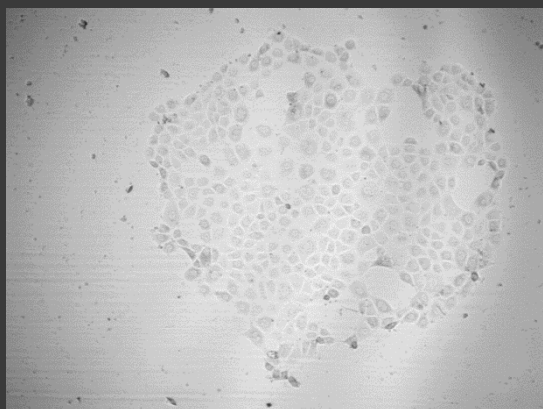
8 bit

16 bit



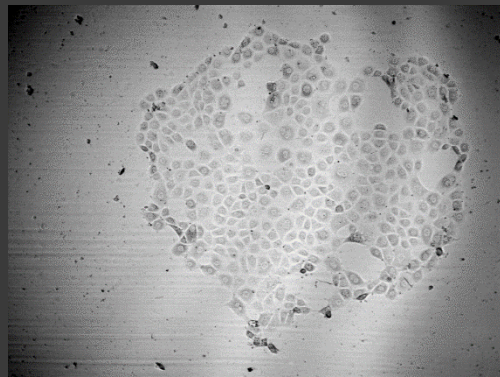
HISTOGRAM – REKONSTRUKCE OBRAZU

Histogram

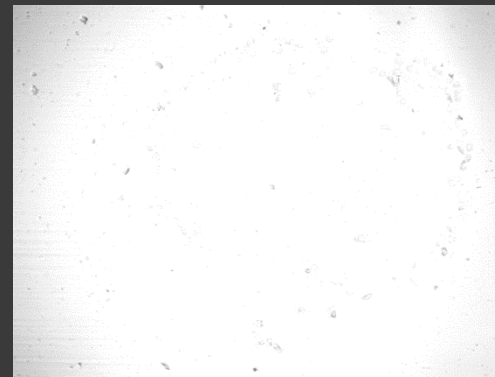
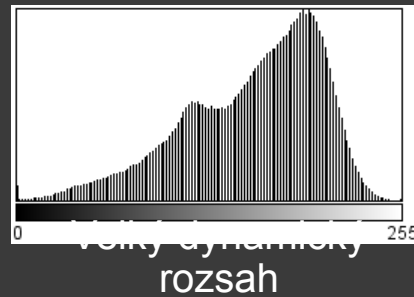


ImageJ:
Image → Adjust
→
Brightness/Contrast

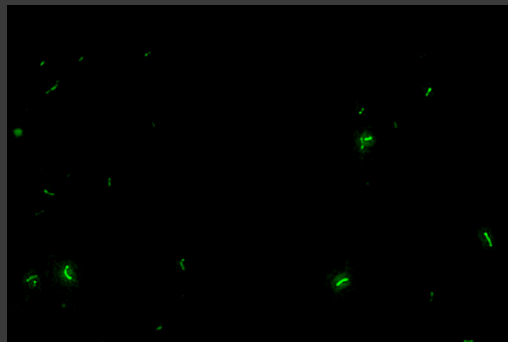
HISTOGRAM – DYNAMIC ROZSAH



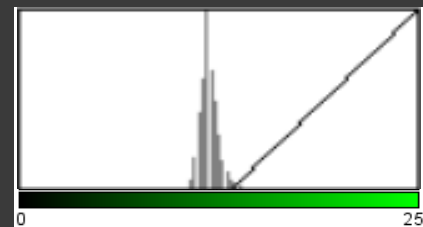
Histogram
Original



Krátká expozice



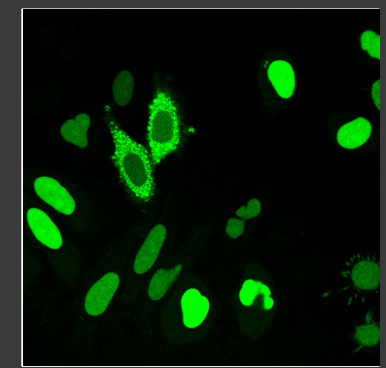
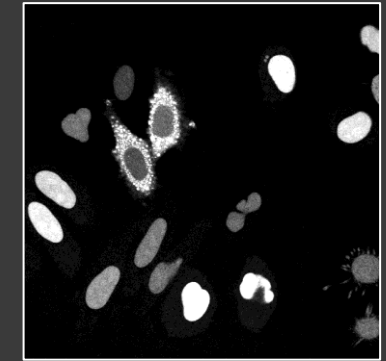
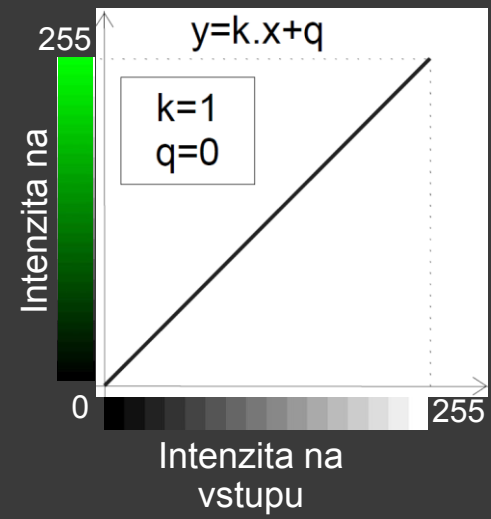
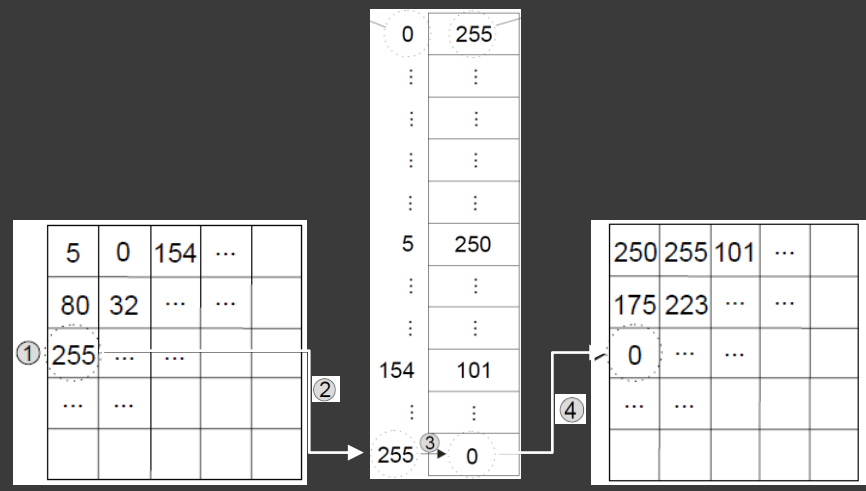
Odstření plozadí



ImageJ:
Image →
Lookup Tables
→ Invert
LUT/Green

HISTOGRAM & LUT – LOOKUP TABLE

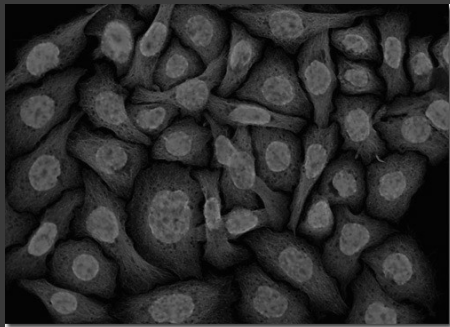
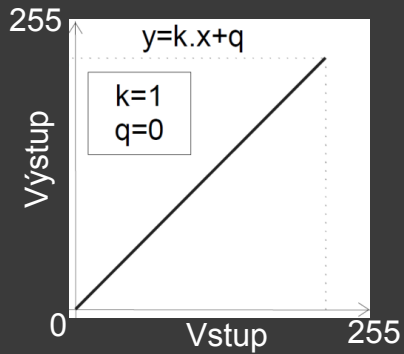
Jak LUT funguje



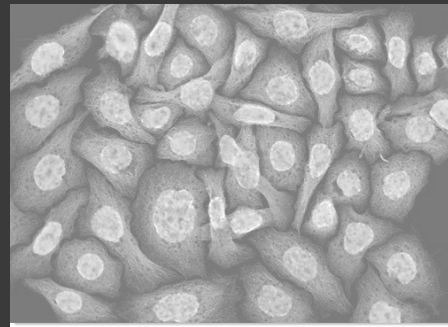
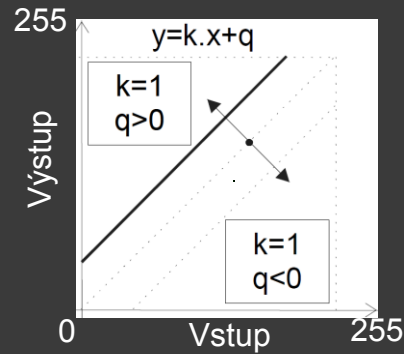
ImageJ:
Image → Adjust
→
Brightness/Contrast

HISTOGRAM & LUT – PŘÍKLADY

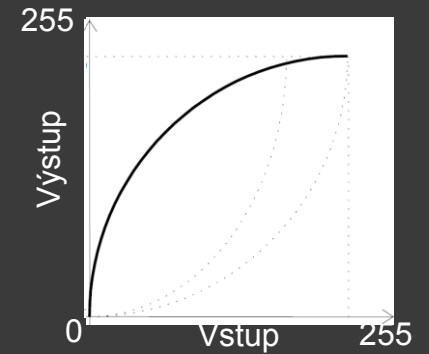
Žádná změna



Jas

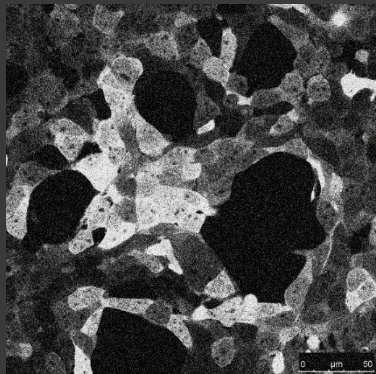


Gama korekce

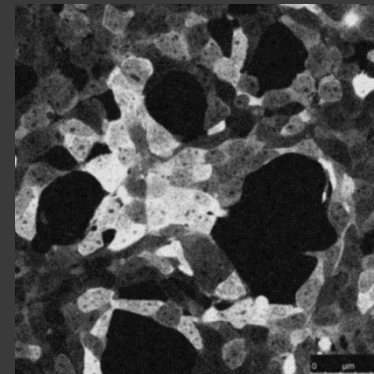
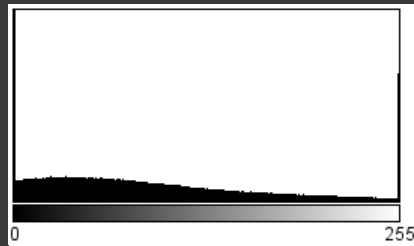


ImageJ:
Process → Filters
→ Gaussian
Blur/Median

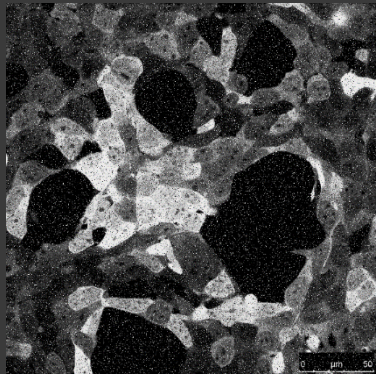
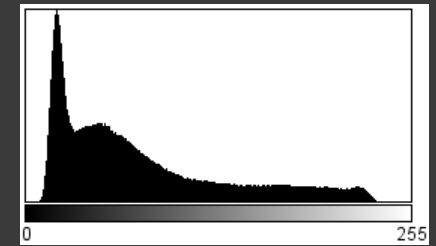
HISTOGRAM - ŠUM



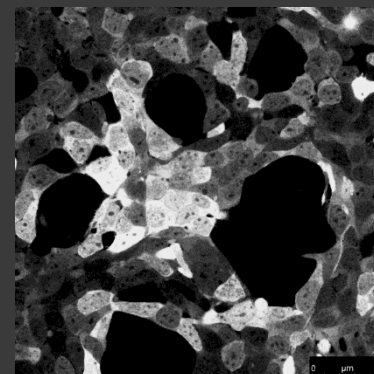
Gaussovský



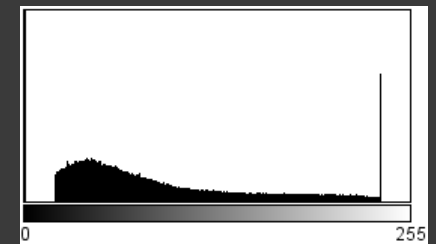
Blur filtering



Sůl & Pepř



Median filtering



DATOVÉ FORMÁTY

Rastrové obrazy

Tvar reprezentovaný maticí pixelů

Pixelace

Fotorealistické obrazy

Typické formáty: JPEG, GIF, TIFF, PNG

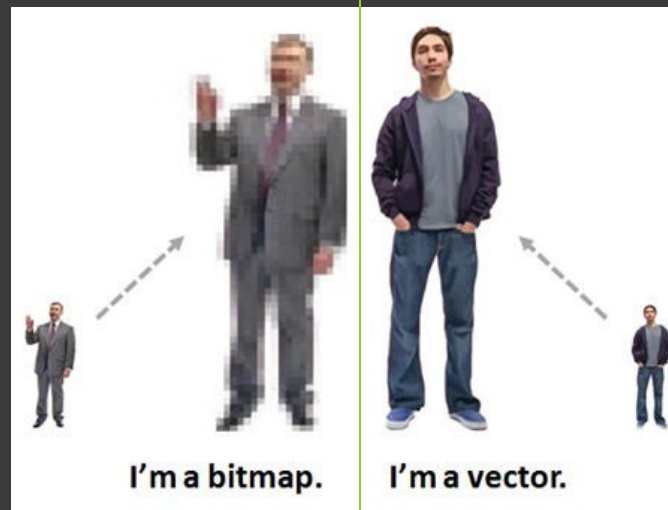
Vektorové obrazy

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)

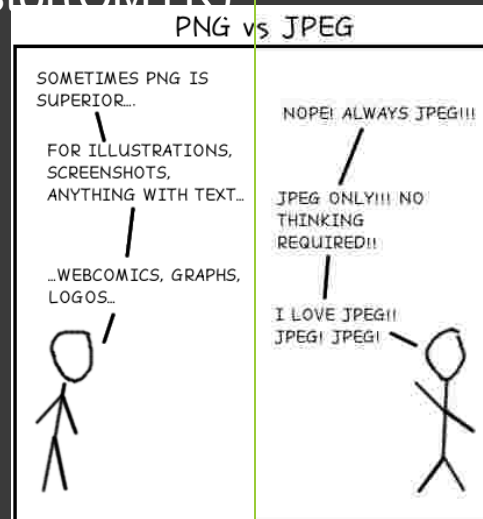
Joint Photographic Expert Group (JPEG)

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

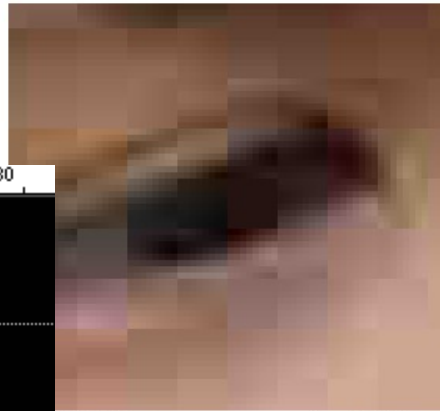
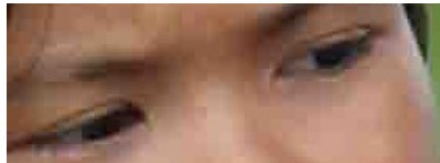
Nepodporuje transparentnost

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.



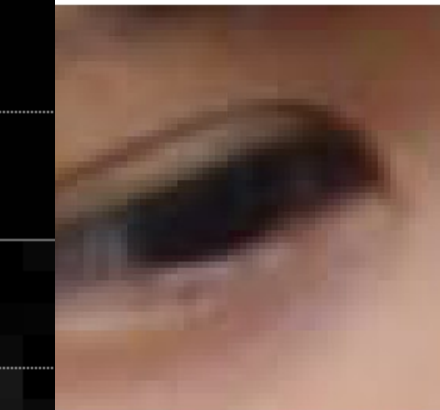
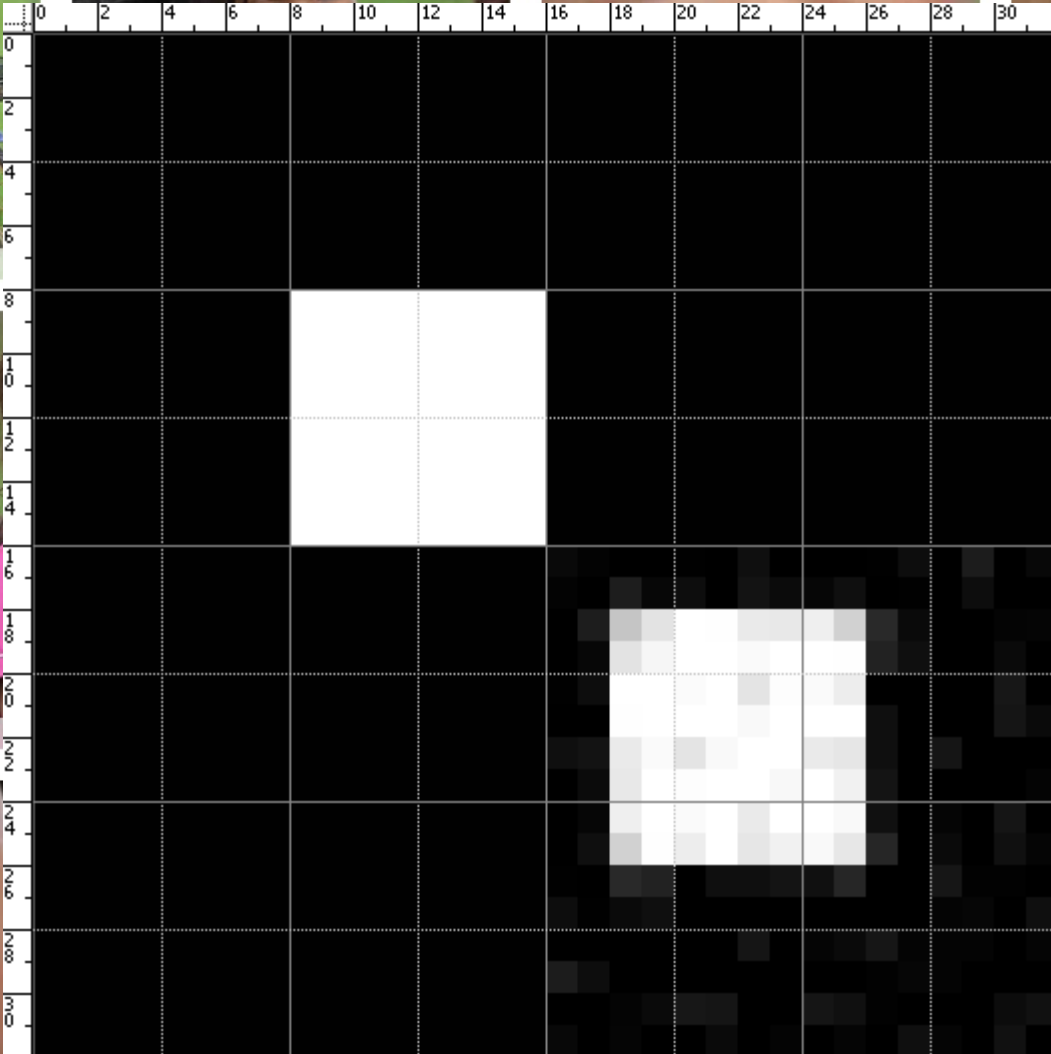
6,25% jpeg compression 0/12



400% jpeg compression 0/12



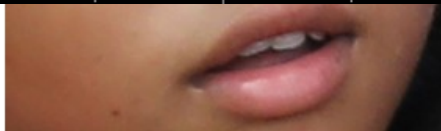
25% jpeg compression 3/12



400% jpeg compression 6/12



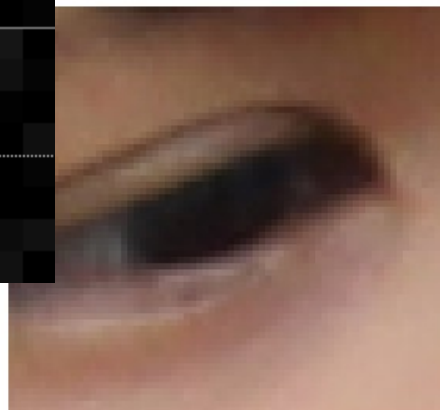
100% jpeg compression 9/12



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



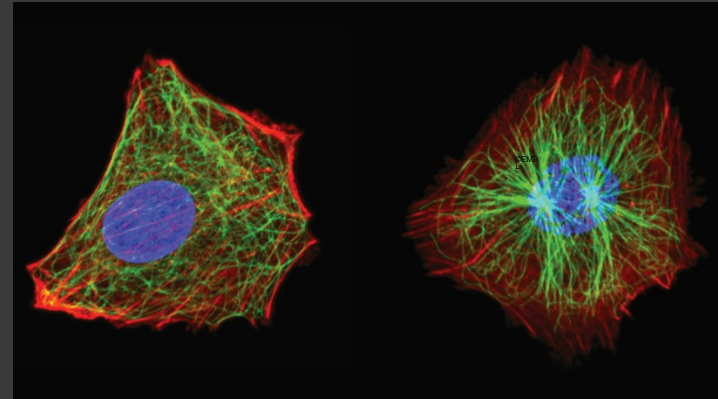
Tagged Image File Format (TIFF)

Fotky, grafika

Možnost bezeztrátové komprese

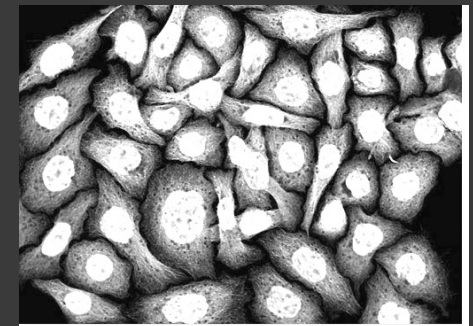
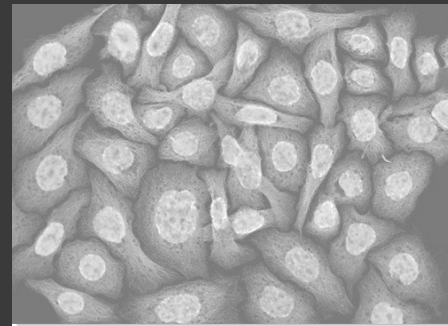
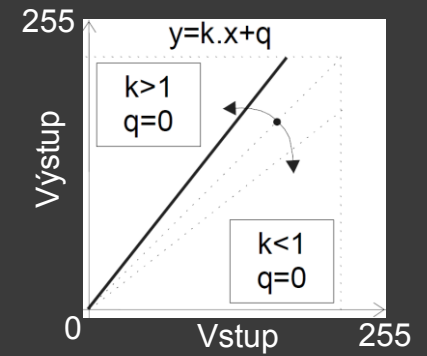
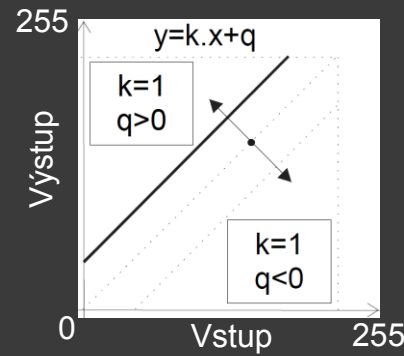
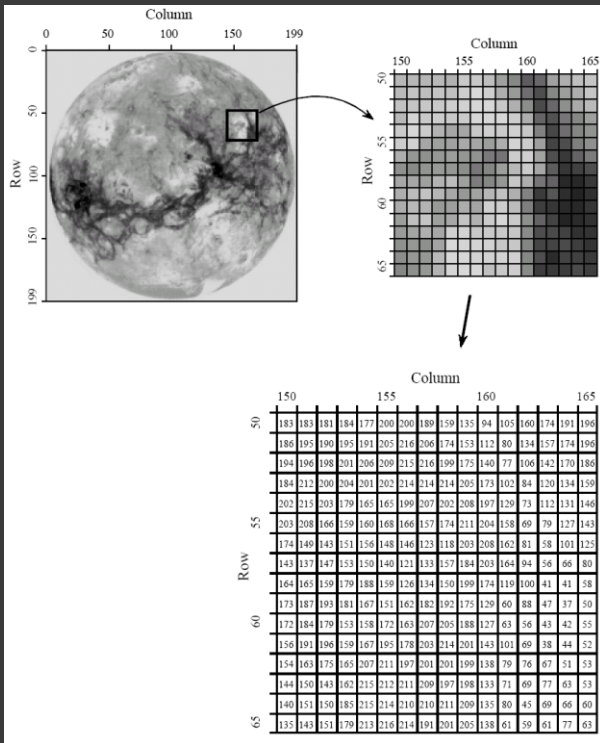
Profesionální, široce podporovaný

Různé barevné prostory, jakékoli rozlišení
a barvy



ImageJ:
Image → Adjust
→
Brightness/Contrast

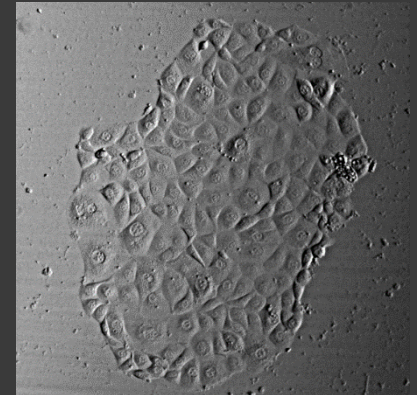
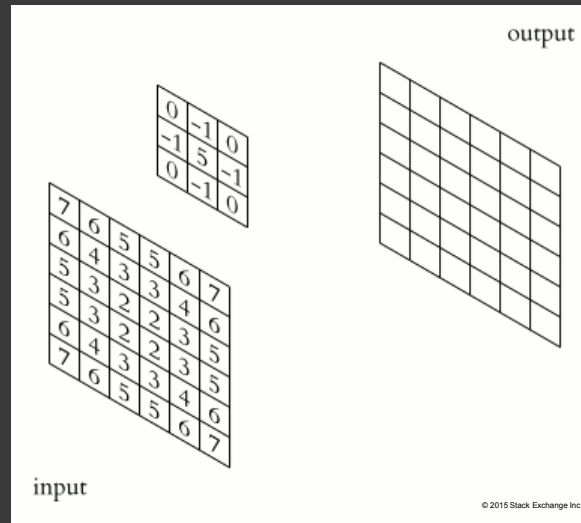
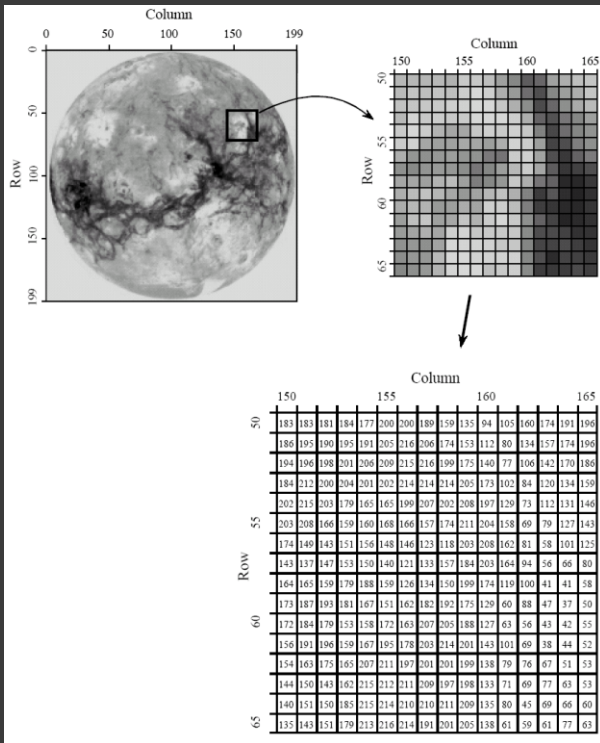
ZPRACOVÁNÍ – BODOVÉ TRANSFORMACE



✖ 30

FILTROVÁNÍ – LOKÁLNÍ ZMĚNY

ImageJ:
Process →
Sharpen/Filters →
Unsharp Mask



ImageJ:

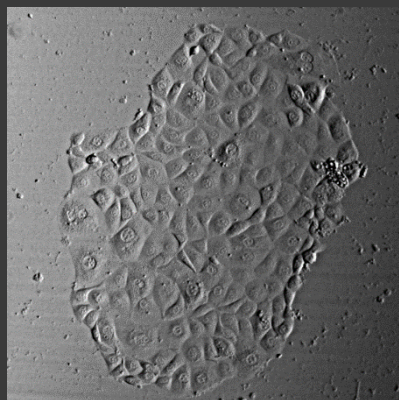
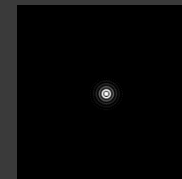
Process →
Sharpen/Smooth/
Find Edges
Plugins → Parallel
Iterative
Deconvolution

FILTROVÁNÍ – PŘÍKLADY

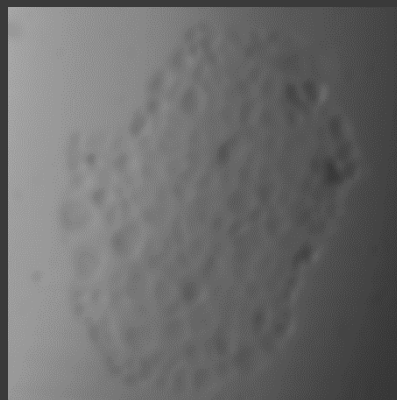
0	0	0	0	0
0	0	-1	0	0
0	-1	5	-1	0
0	0	-1	0	0
0	0	0	0	0

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

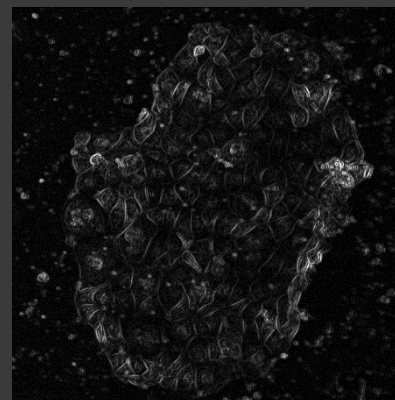
	0	1	0	
	1	-4	1	
	0	1	0	



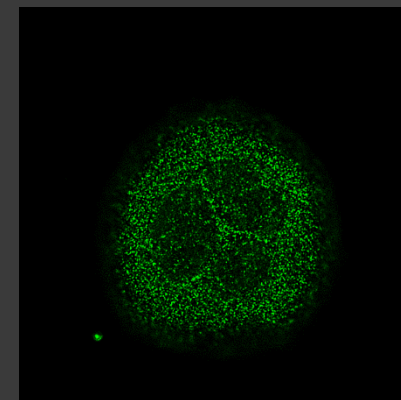
doostření



rozmazání



detekce hran

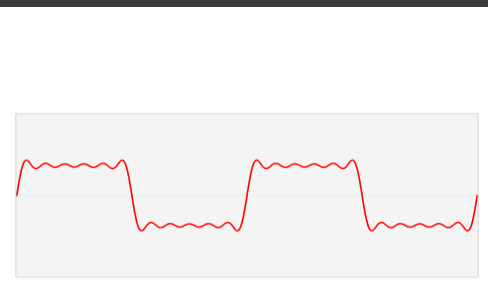
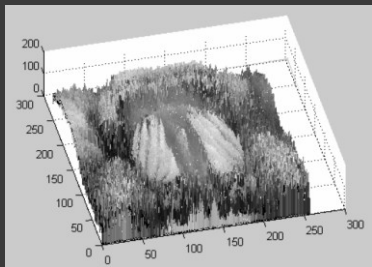
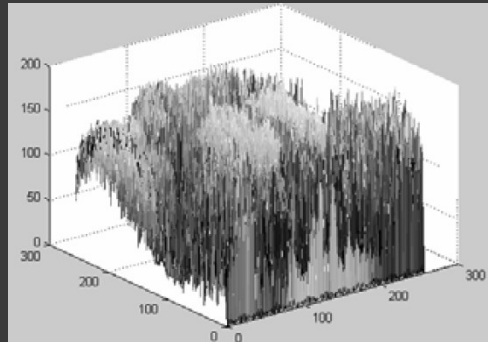
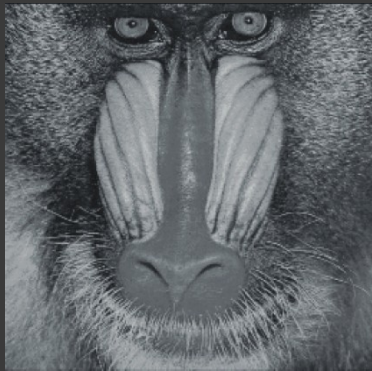


dekonvoluce

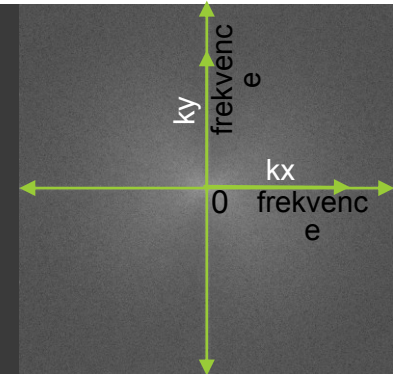
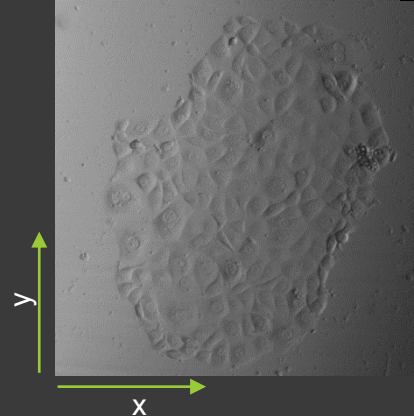
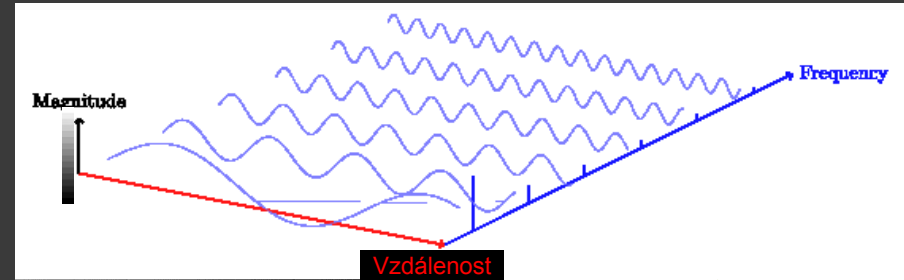
ImageJ:
Process → FFT →
FFT

FILTROVÁNÍ – GLOBÁLNÍ TRANSFORMACE

Fast Fourier Transformation

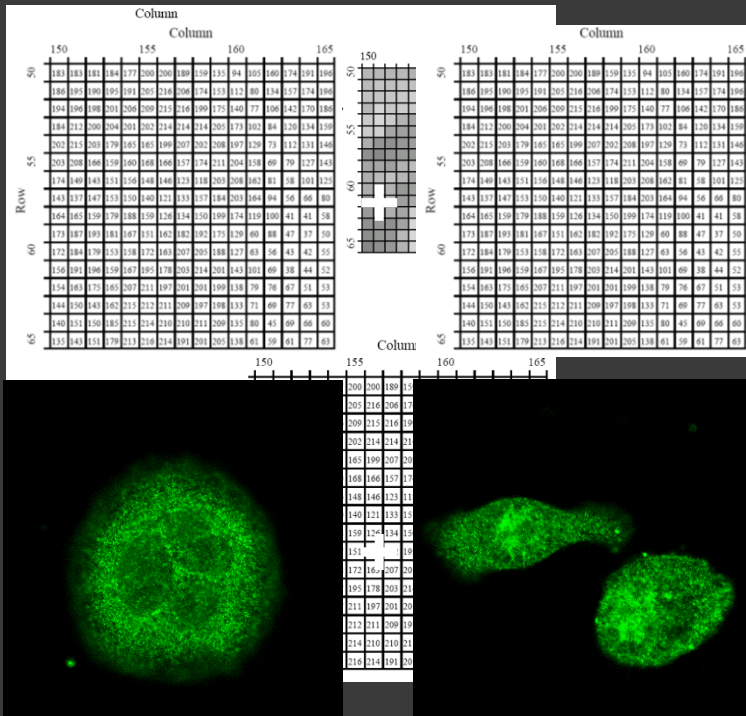


Konvoluce = Násobení



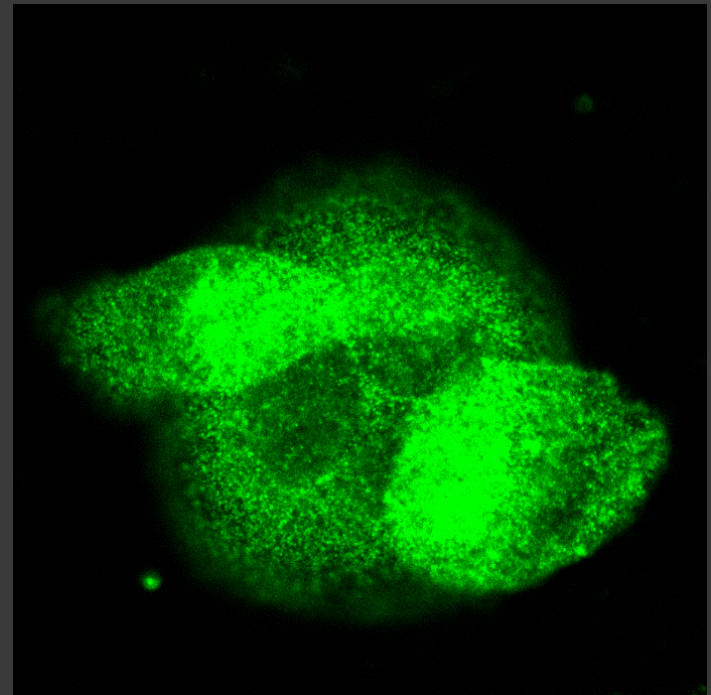
OBRAZOVÁ ARITMETIKA

Sčítání



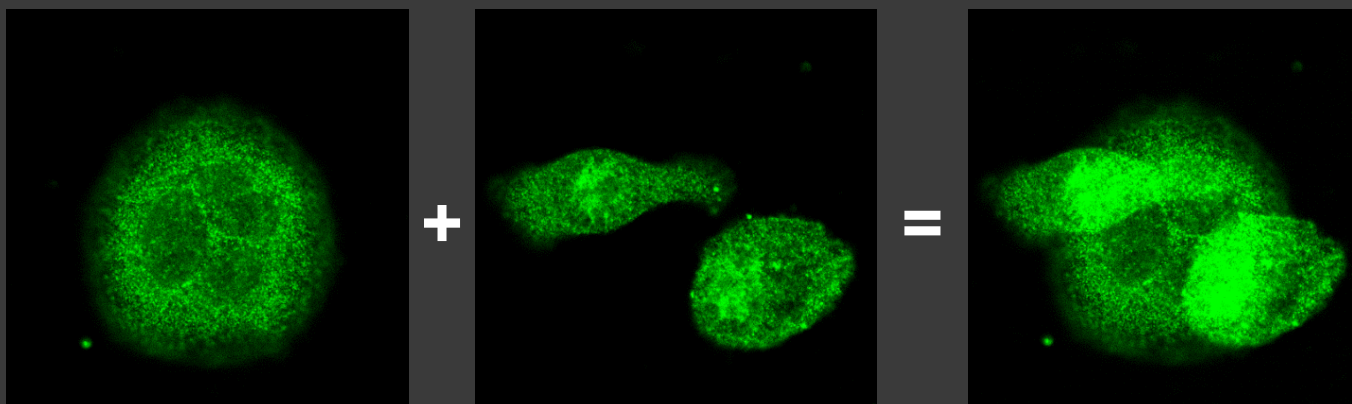
==

==

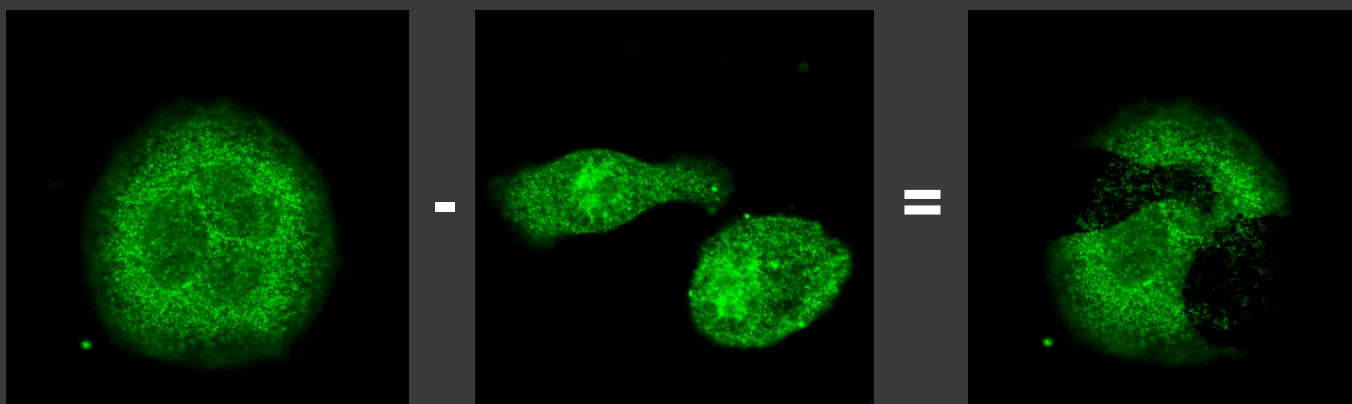


OBRAZOVÁ ARITMETIKA

Sčítání

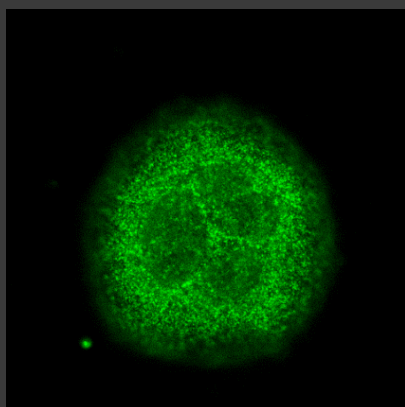


Odčítání

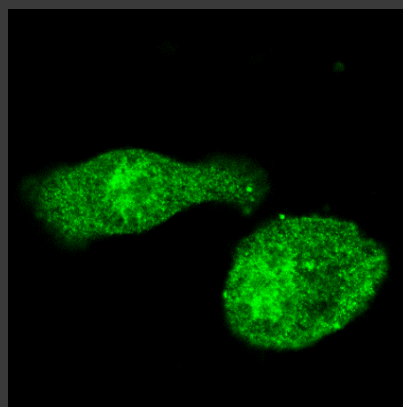


OBRAZOVÁ ARITMETIKA

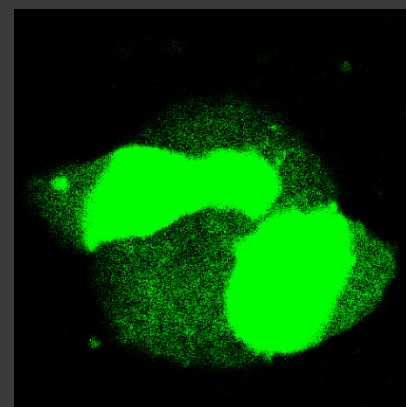
Násobení



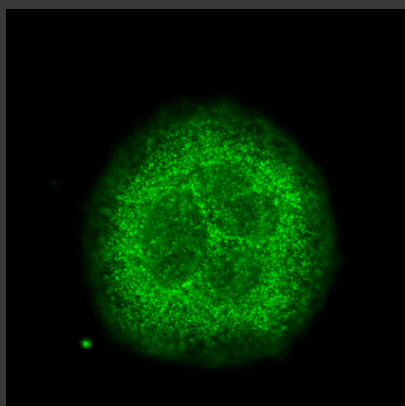
x



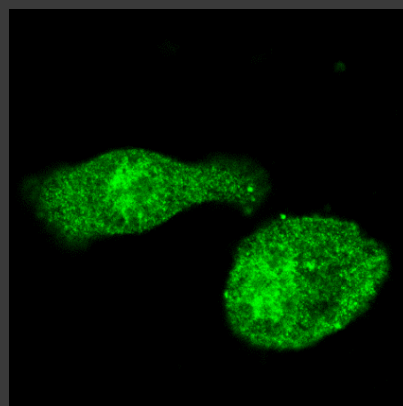
=



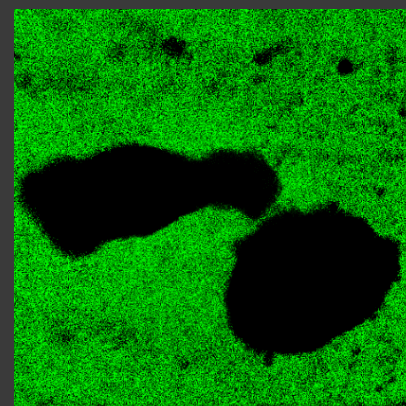
Dělení



/

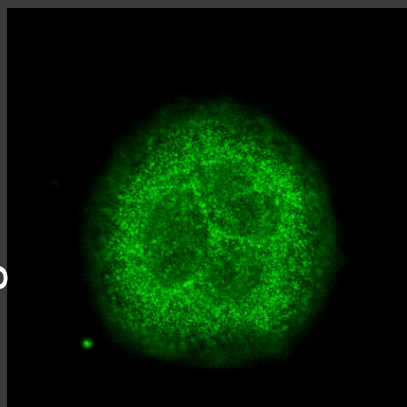


=

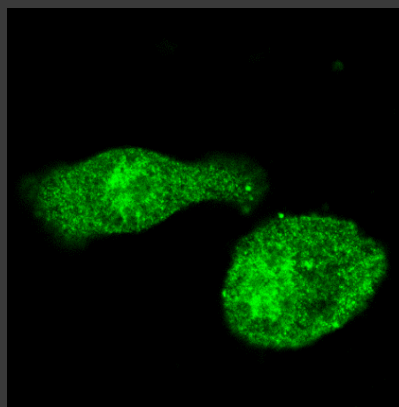


OBRAZOVÁ ARITMETIKA

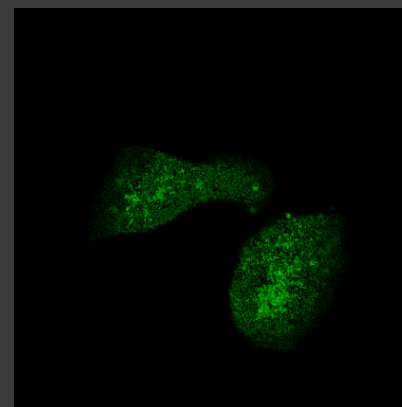
Logické AND



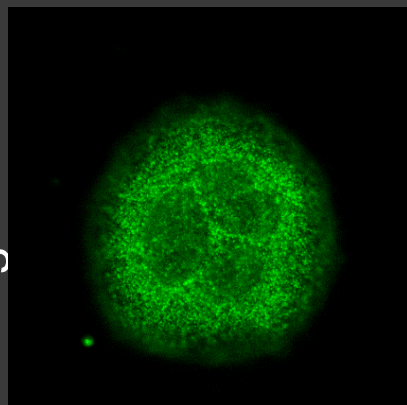
AND



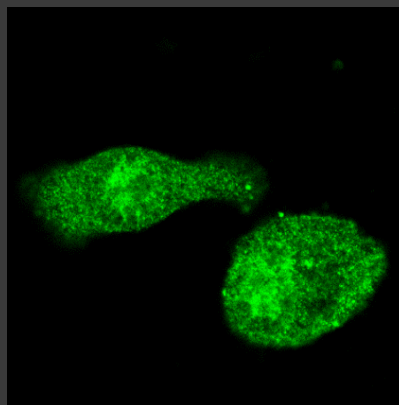
==



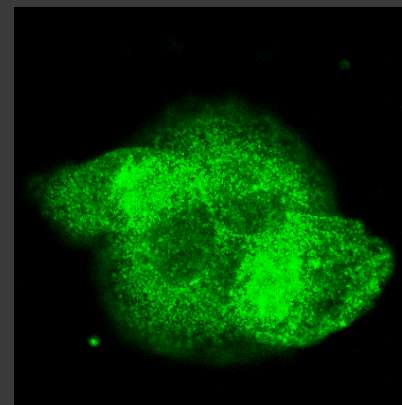
Logické OR



OR



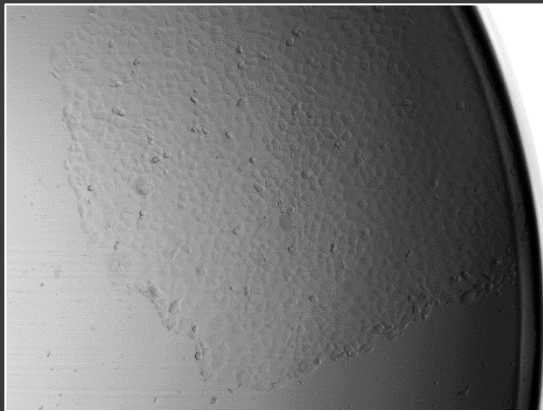
==



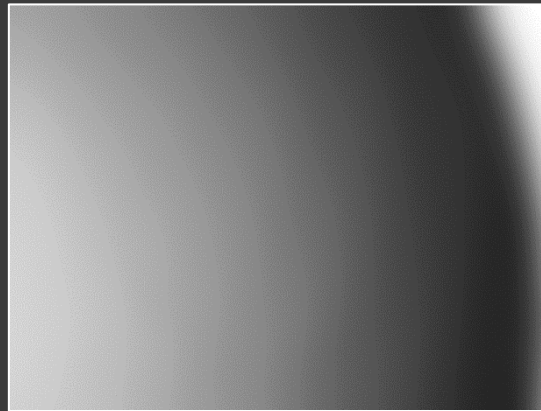
OBRAZOVÁ ARITMETIKA – PŘÍKLADY

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

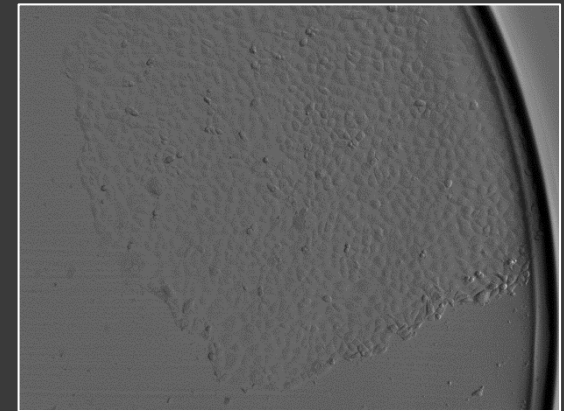
Flat Field korekce



/



=

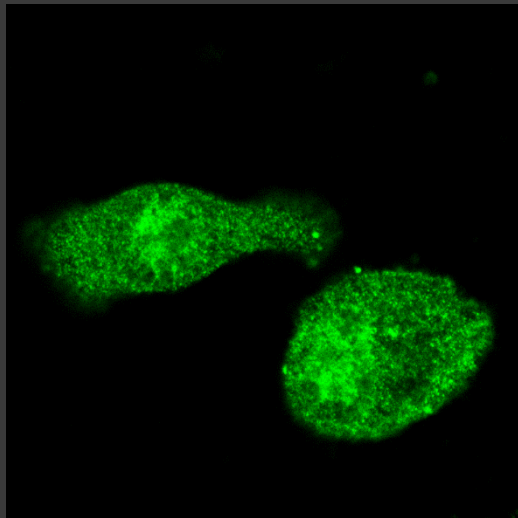


Gaussian blur

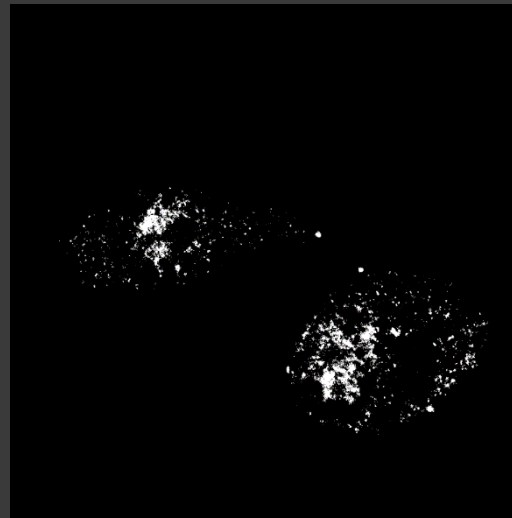
OBRAZOVÁ ARITMETIKA – PŘÍKLADY

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

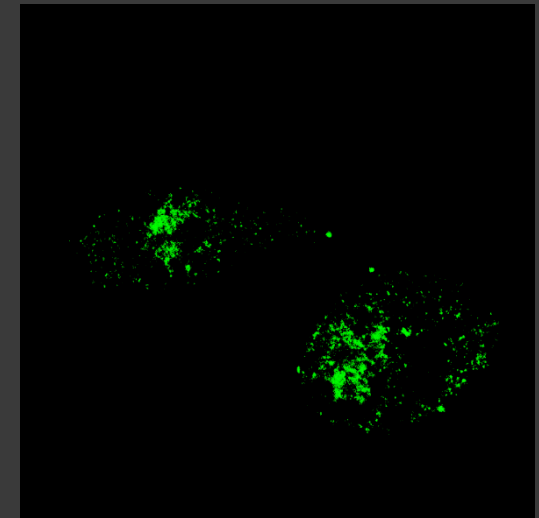
Segmentace



AND



=

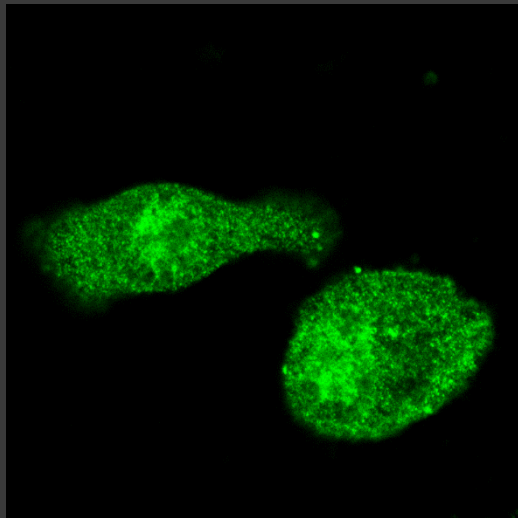


Maska

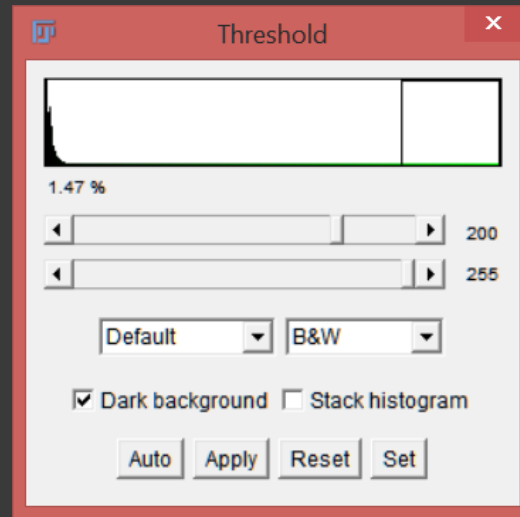
ImageJ:
Image → Adjust
→ Threshold

SEGMENTACE – MANUÁLNÍ PRAHOVÁNÍ TRESHOLDING

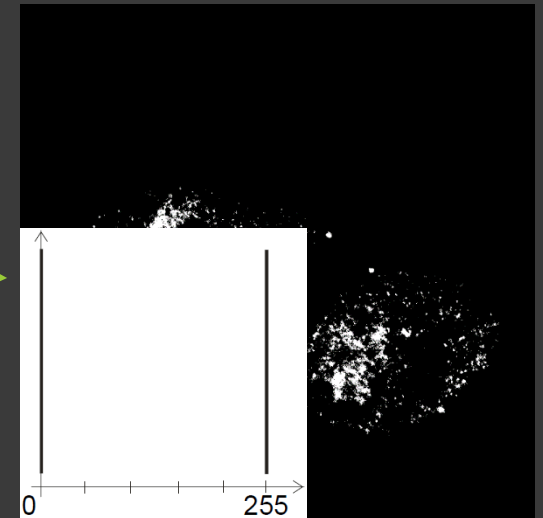
Originální obraz



Prahování



Maska



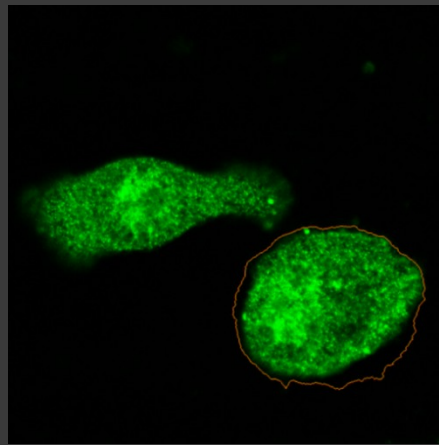
ImageJ:
Plugins →
Segmentation →
Level Sets

SEGMENTACE – POLOAUTOMATICKÁ

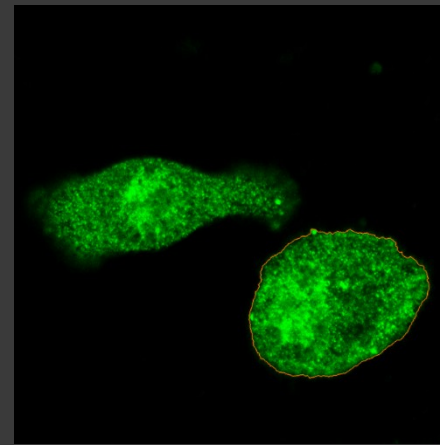
Start - ROI



Level sets



Konec



Maska



ImageJ:
Plugins →
Segmentation →
Level Sets

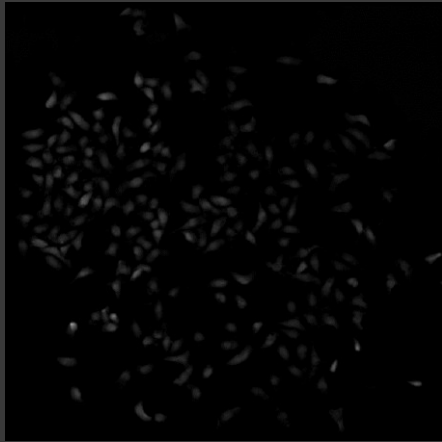
SEGMENTACE – POLOAUTOMATICKÁ



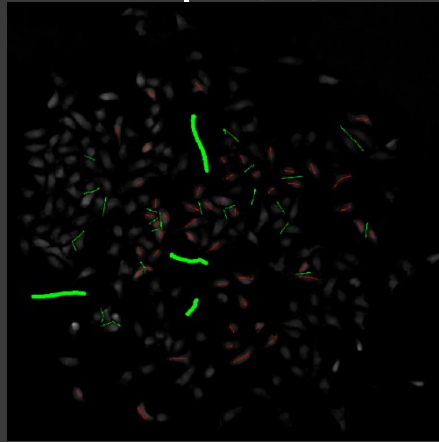
SEGMENTACE – STROJOVÉ UČENÍ

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

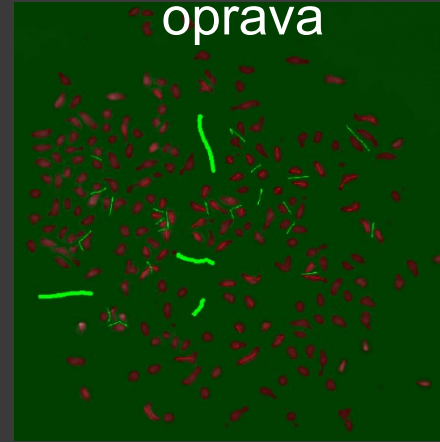
Originální obraz



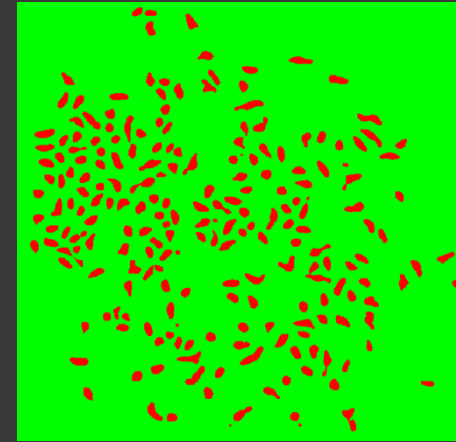
Učení



Kontrola a
oprava



Maska



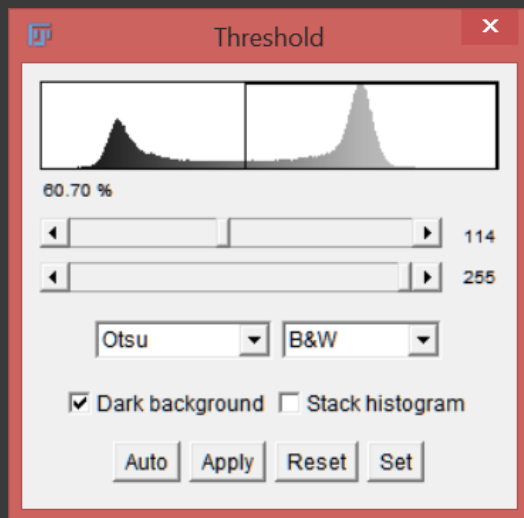
SEGMENTACE – AUTOMATIZOVANÁ

ImageJ:
Image → Adjust
→ Threshold →
Otsu

Originální obraz

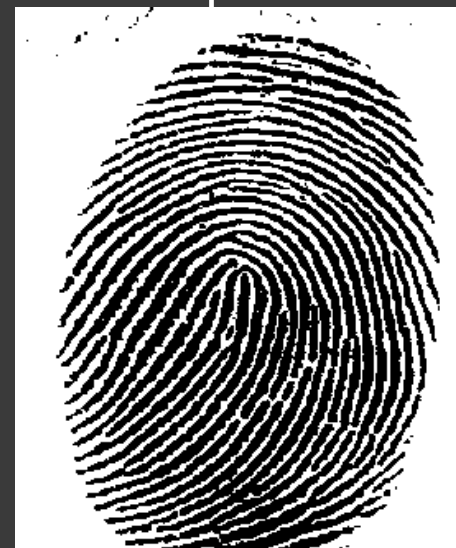


Otsu - Klastrování



Intra-class variance
minimal

Segmentovaný



ZMĚNA MORFOLOGIE MASKY

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

Roztažení



+ Structuring
element



Otevření



Eroze



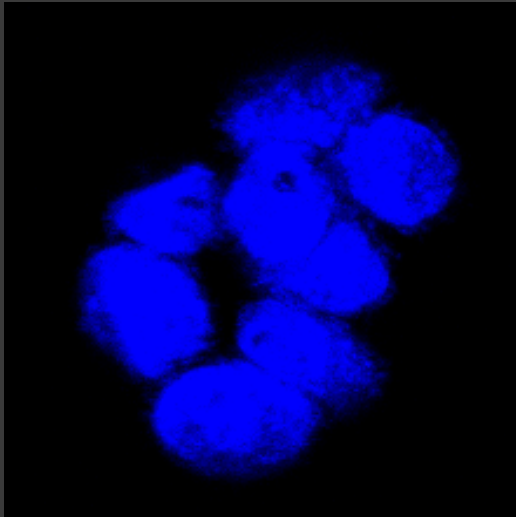
Zavření



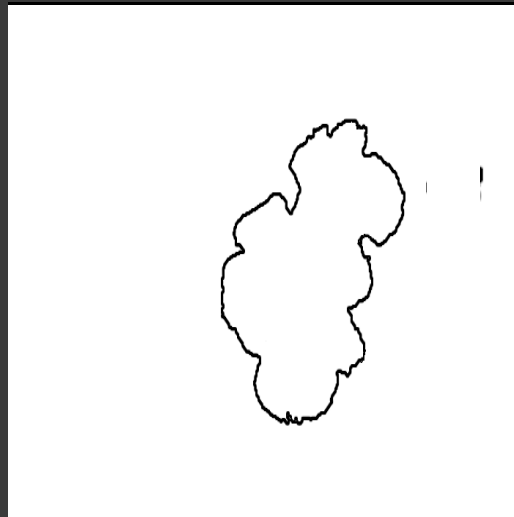
ZMĚNA MORFOLOGIE MASKY

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

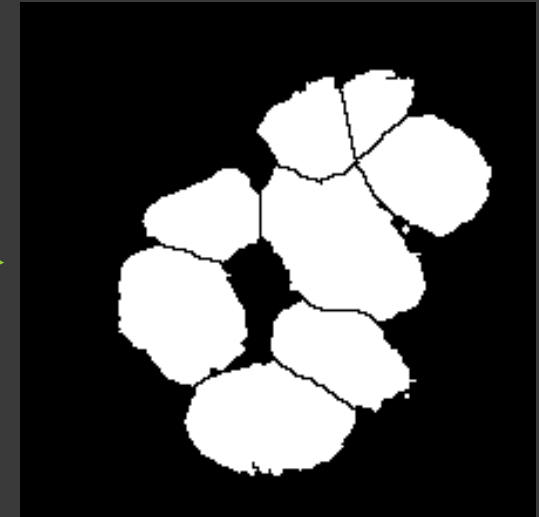
Originální obrazy



Prahování



Watershed

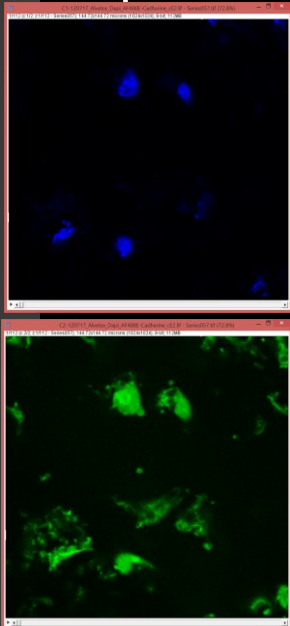


Voda stéká do údolí

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

SEGMENTACE – 3D MODELOVÁNÍ

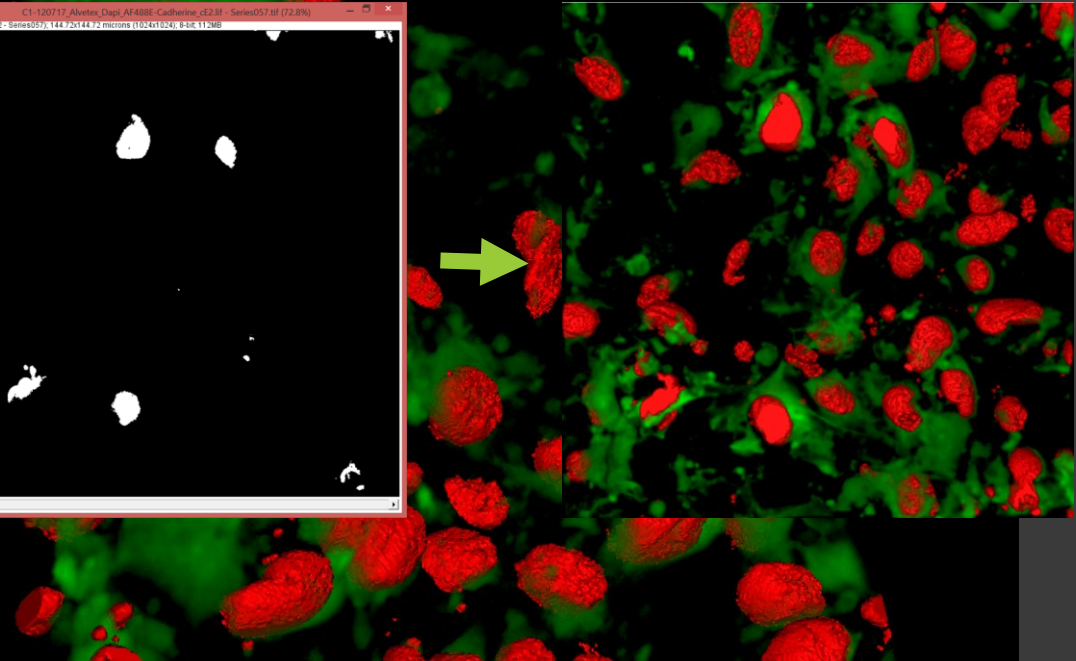
Originální



Oprahovaná jádra



3D projekce



ANALÝZA - SOFTWARE

CellProfiler

MD Metaxpress

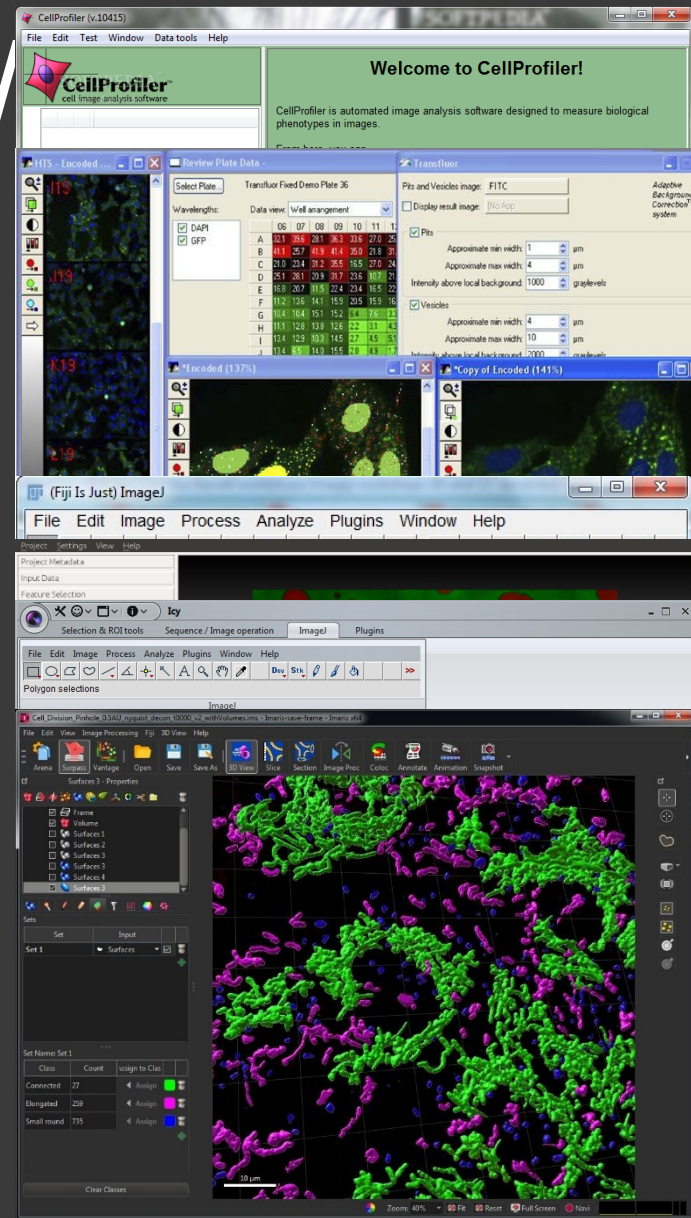
LAS X

FIJI / ImageJ

Ilastik

Icy

Imaris



ImageJ:
Image →
Properties
Analyze →
Analyze Particles

OBRAZOVÁ ANALÝZA - STEREOLOGIE

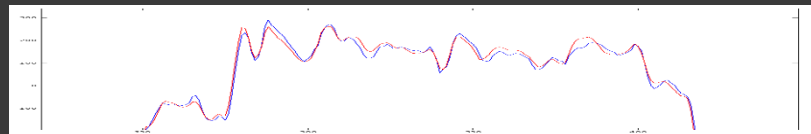
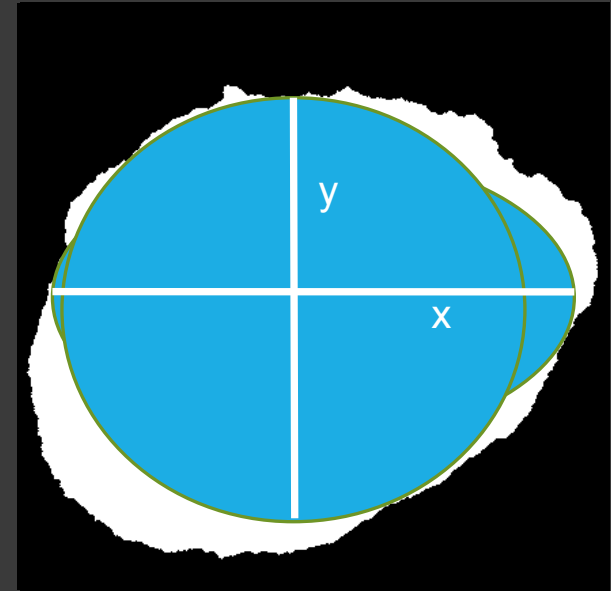
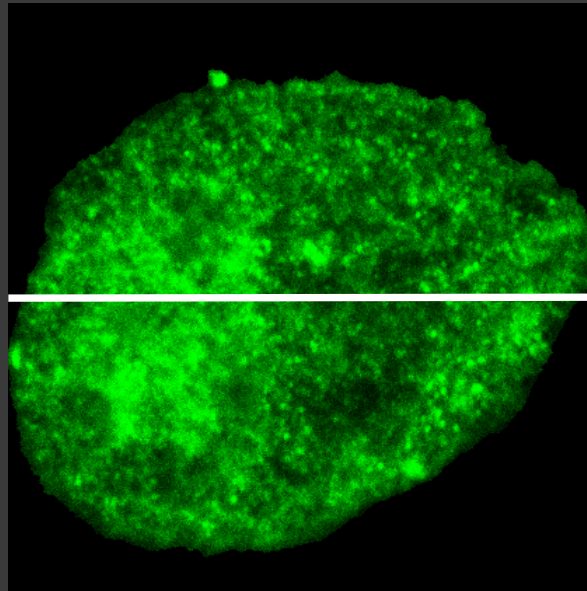
Velikost pixelu

Plocha

Intenzita (bitová hl.)

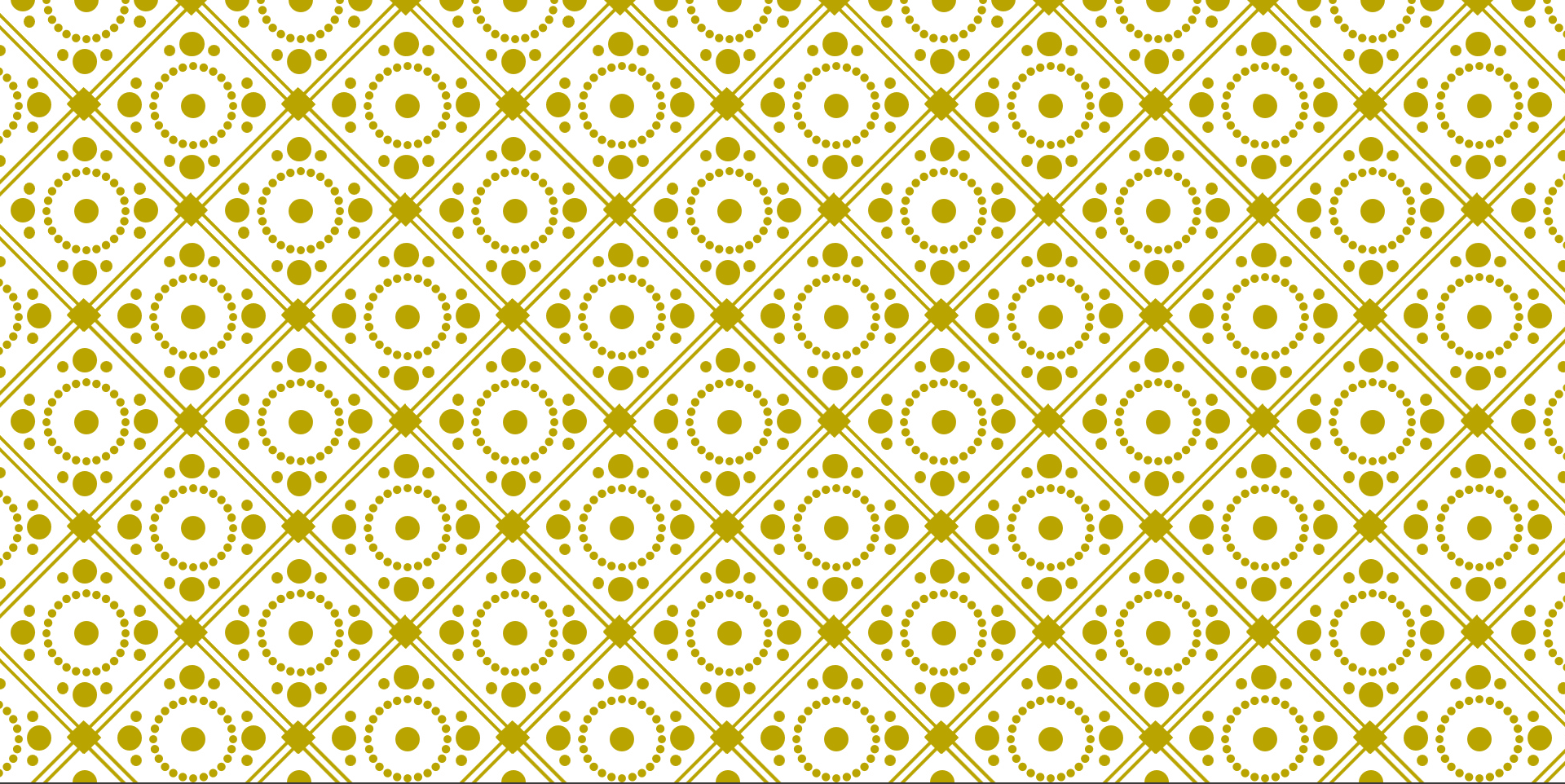
Textura

Morfologie



PROBLÉMY

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

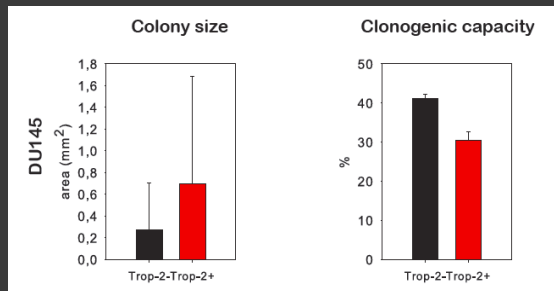
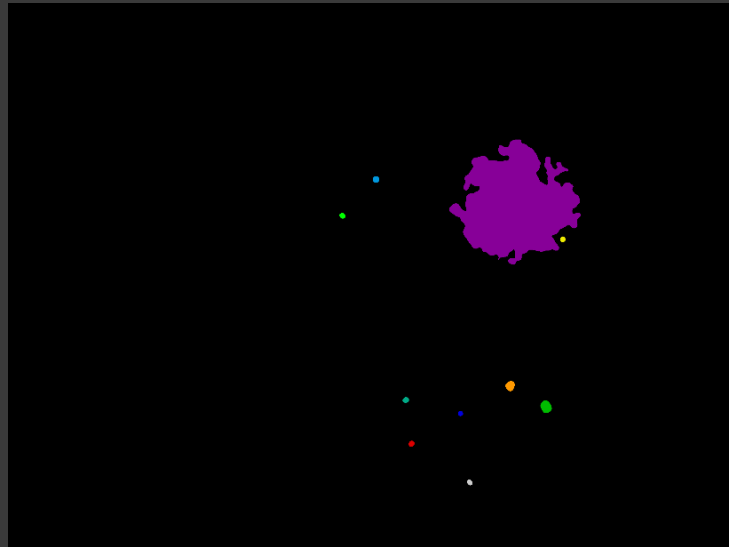


APLIKACE

Příklady analýz

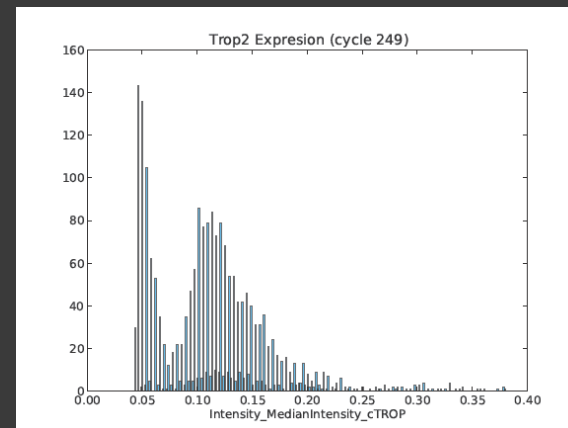
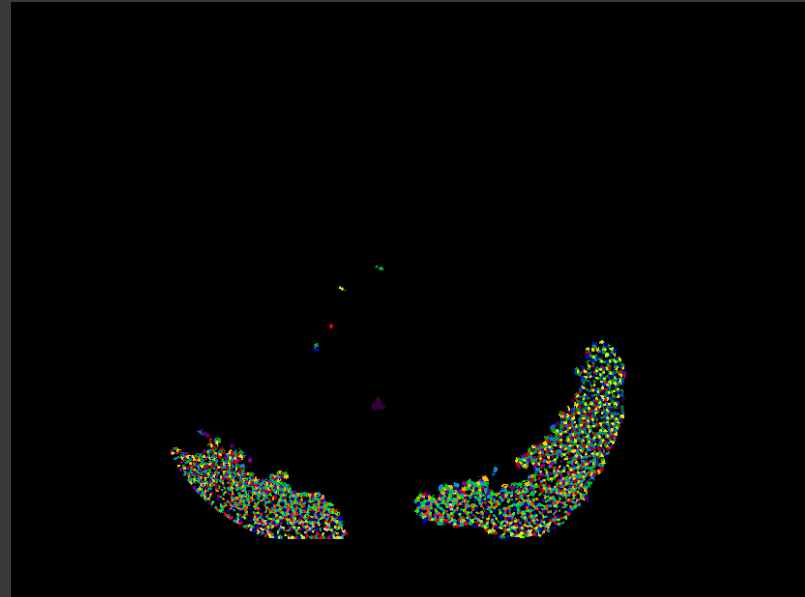
POČÍTÁNÍ BUNĚČNÝCH KOLONIÍ

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

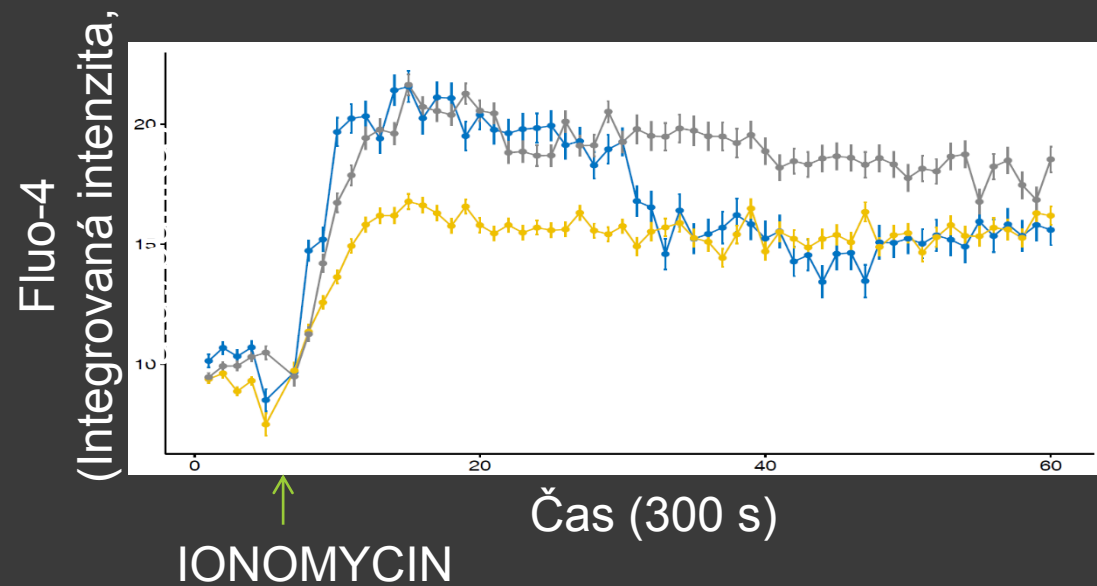
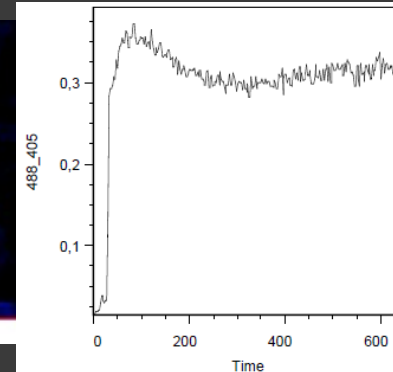
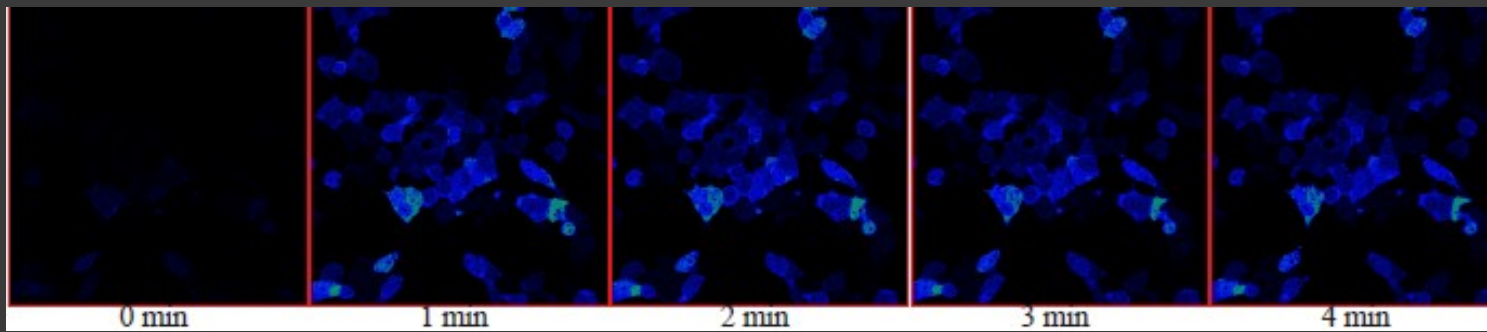


KVANTIFIKACE POVRCHOVÝCH MARKERŮ

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

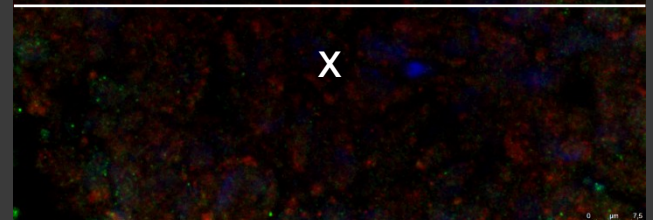
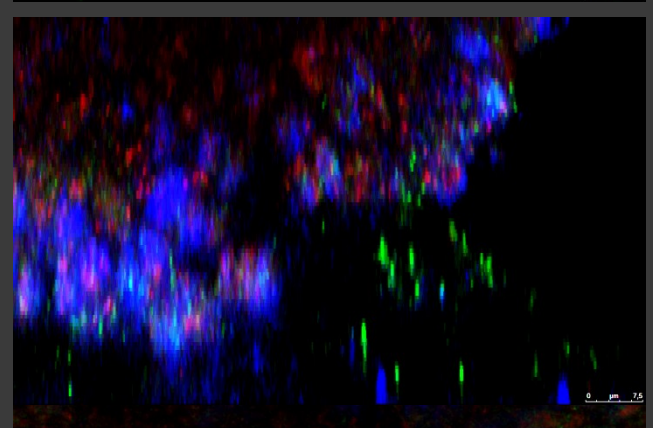
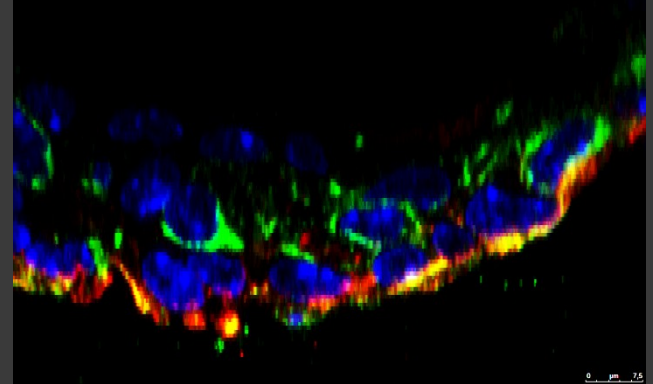
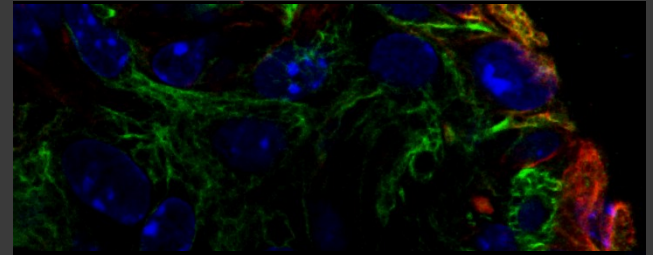
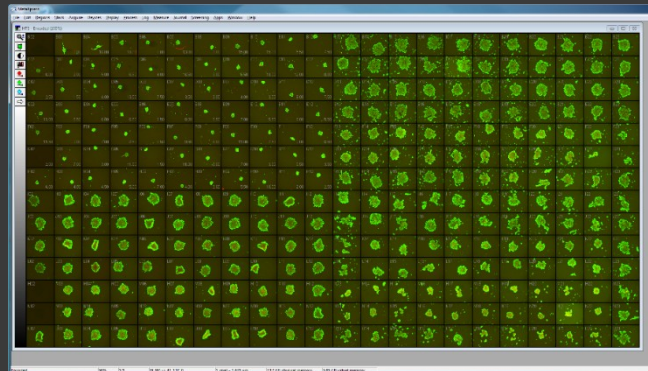
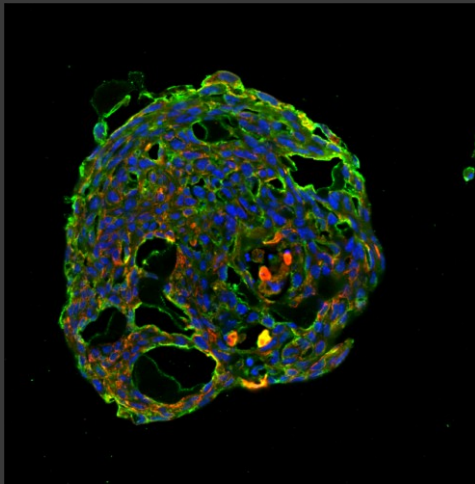
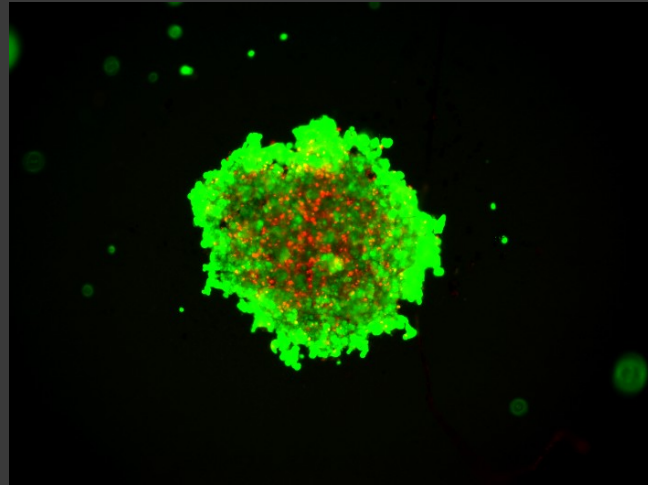
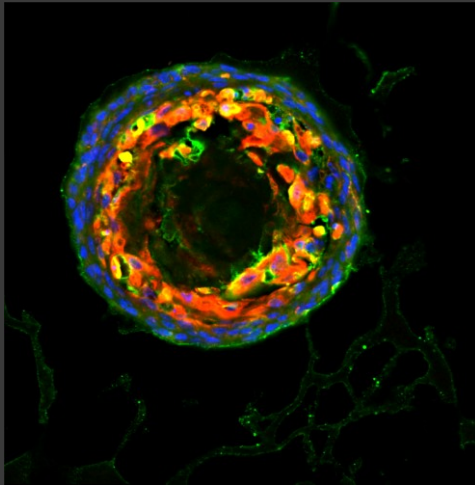


ANALÝZA ŽIVÝCH BUNĚK



3D ANALÝZY

- Kvantifikace objemu
- 3D projekce
- 3D kultivace



Z

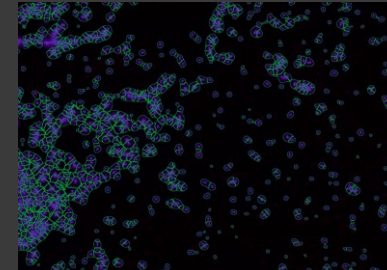
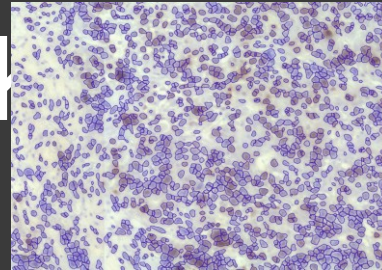
X

DETEKCE SFEROIDŮ A INVAZIVITY

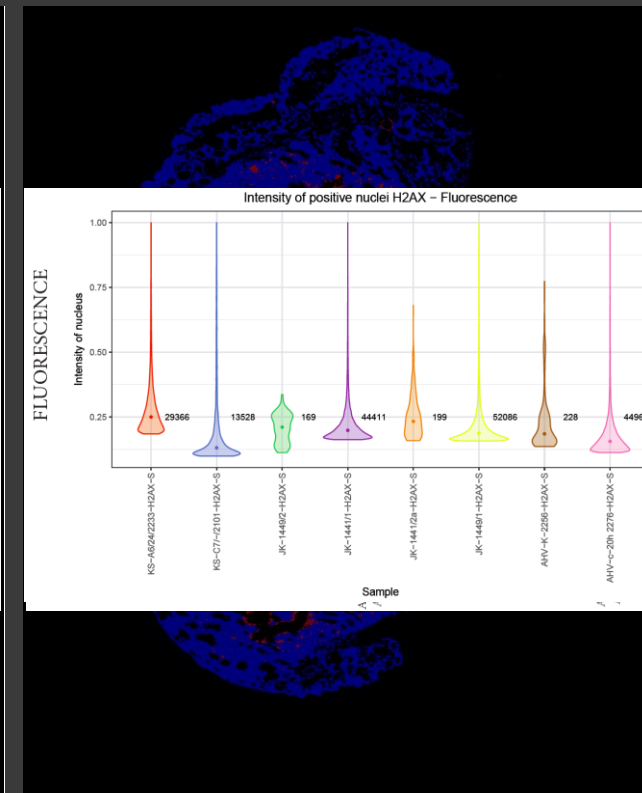
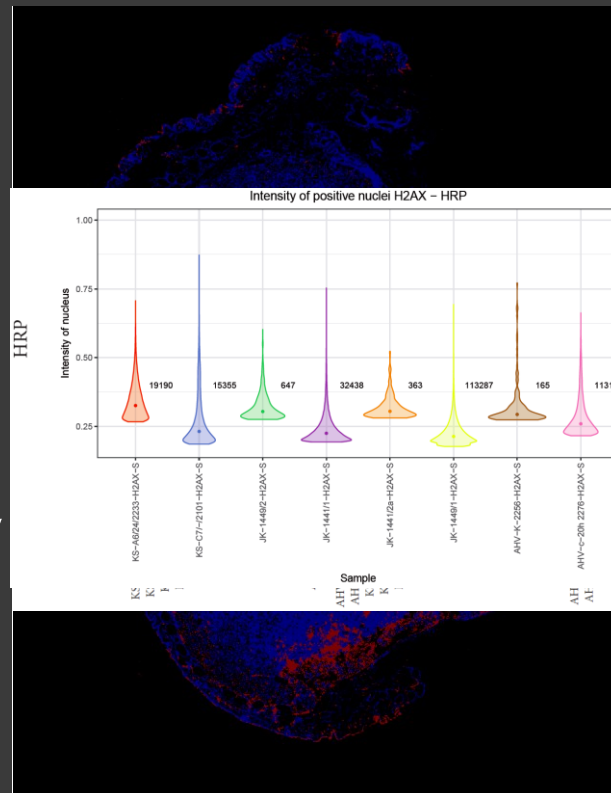
- Hightroughput
- Průchozí světlo
- Detekce sferoidů
- Detekce invadujících buněk
- Plocha



ANALÝZA TR



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



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



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

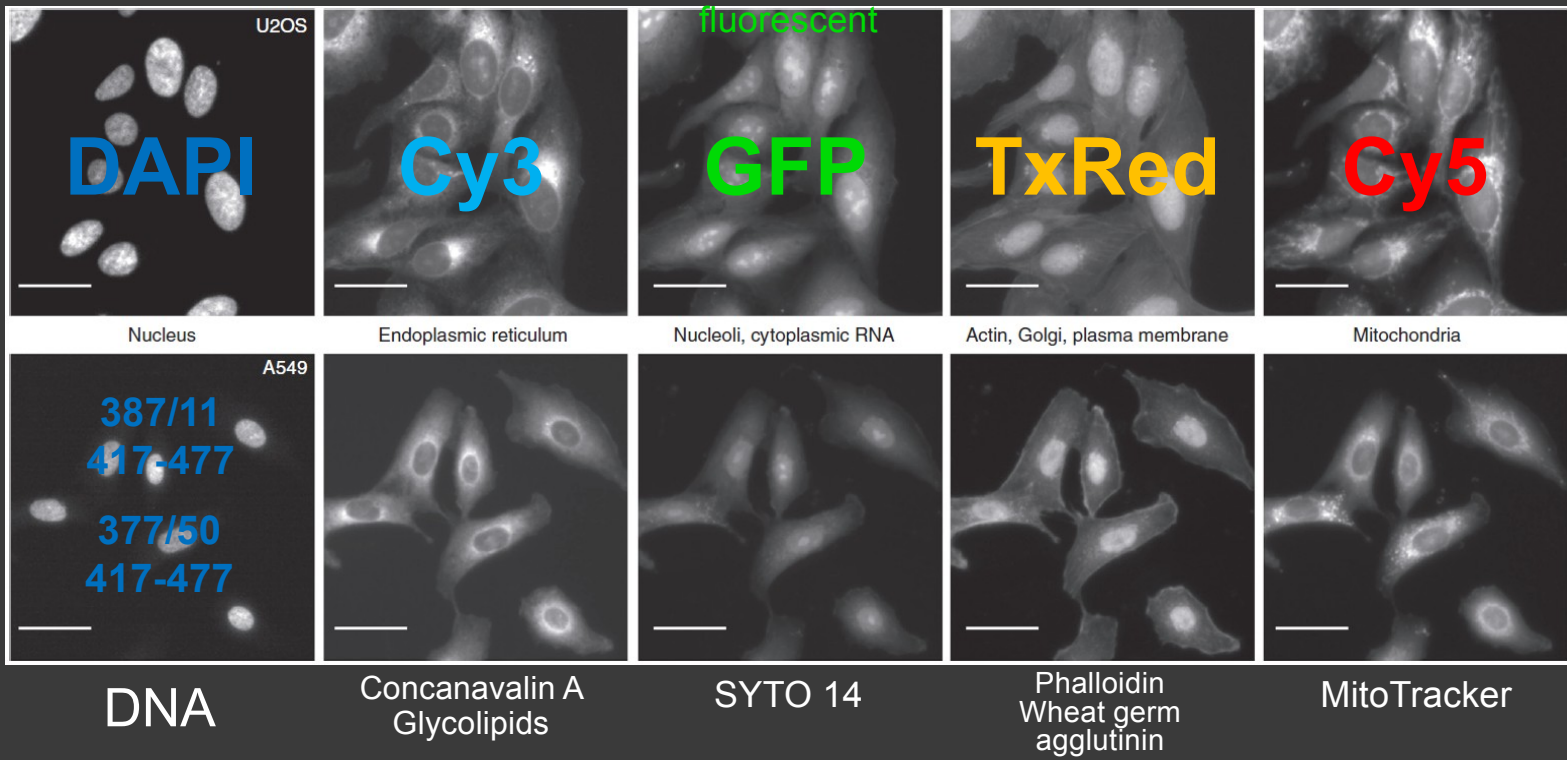
Hoechst 33342

Alexa Fluor 488

Green

AF568, AF555

Deep Red



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



Výsev buněk



Pátek

4 h

Speciální deska

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

Značení



Pondělí

3 h

Zavedený
protokol

Poloautomatické

Snímání



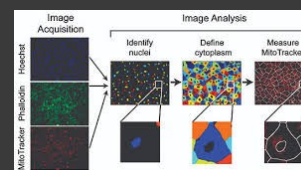
Pondělí

40 h

Zavedený
protokol

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

Analýza obrazů



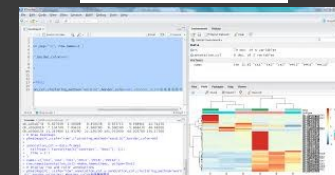
Čtvrtek

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 - VÝSTUPY

Obrázky

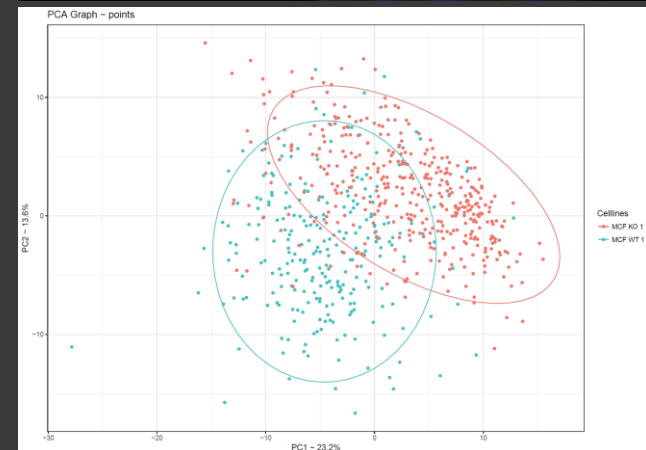
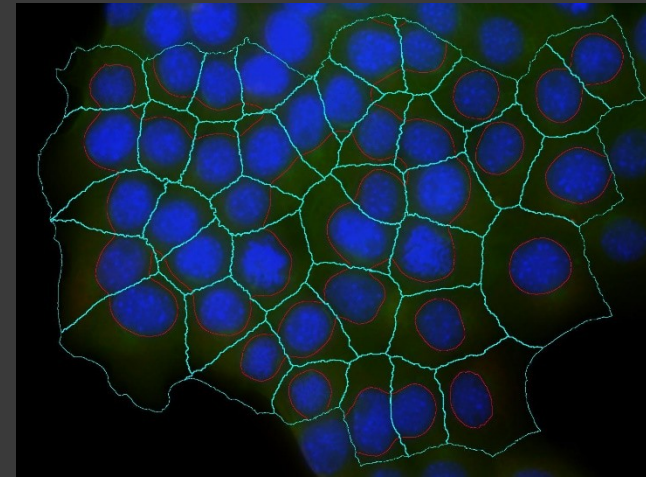
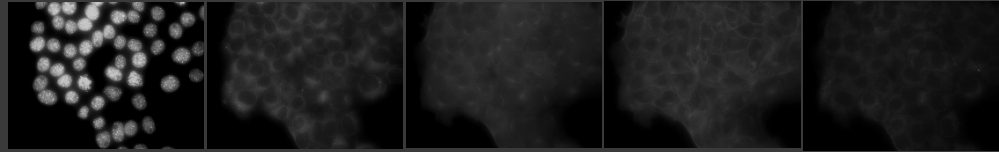
- 16bit a 12bit šedotónové
- Kontrolní obrazy segmentace
- 240GB dat – 1 opakování

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 - OBRAZKY

DAPI

Cy3

GFP

TxRed

Cy5

Hoechst 33342

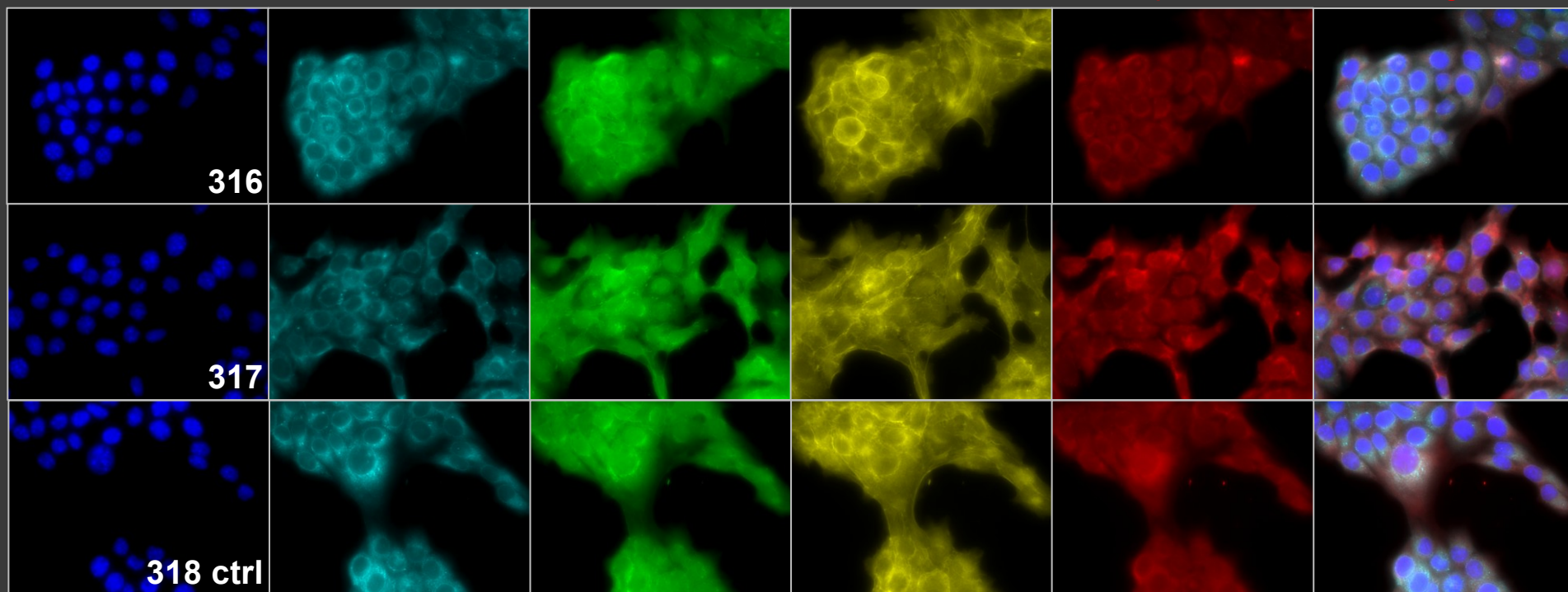
Alexa Fluor 488

Green

AF568, AF555

Deep Red

Merge



316

317

318 ctrl

DNA

Concanavalin A
Glycolipids

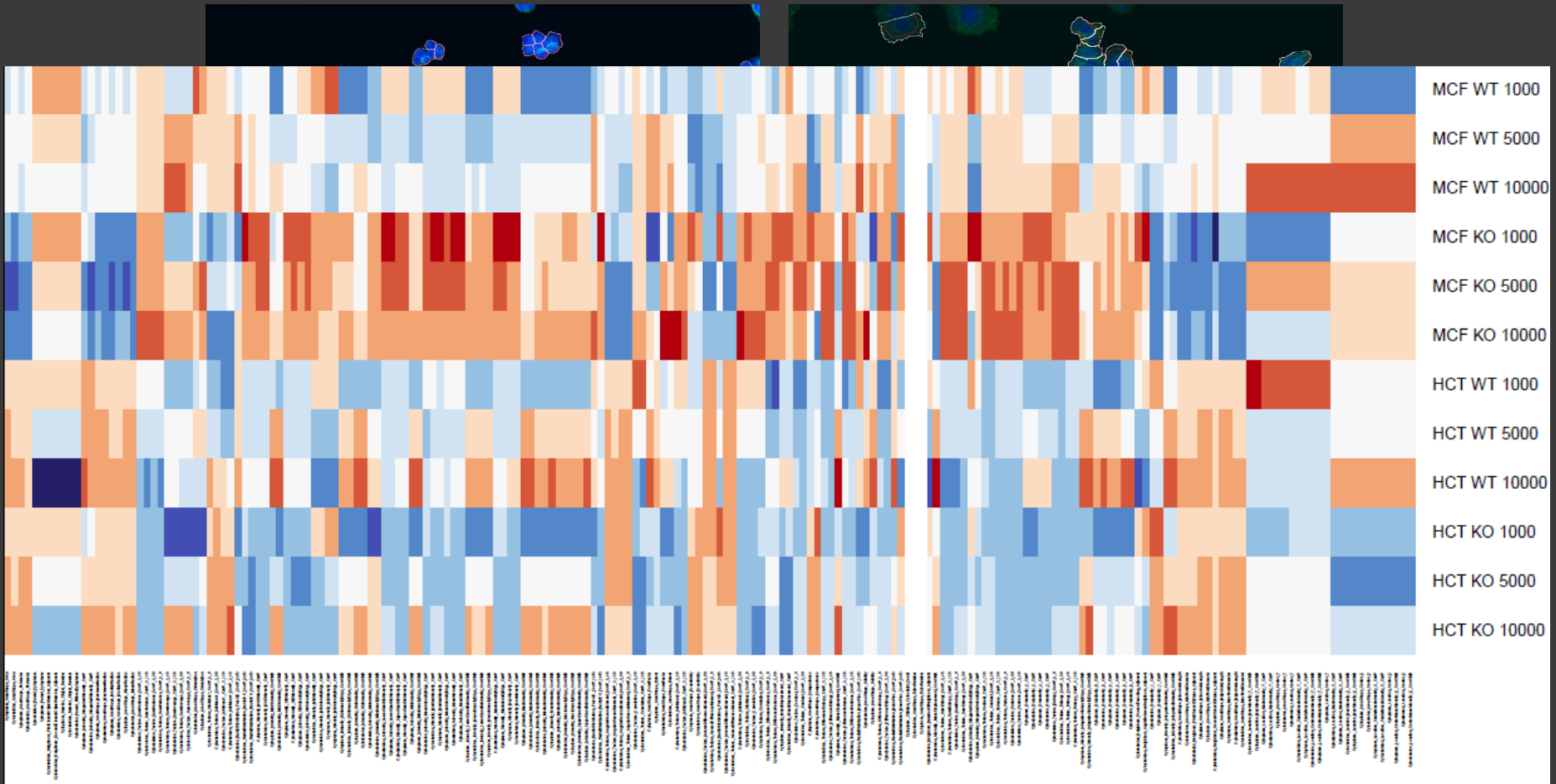
SYTO 14

Phalloidin
Wheat germ
agglutinin

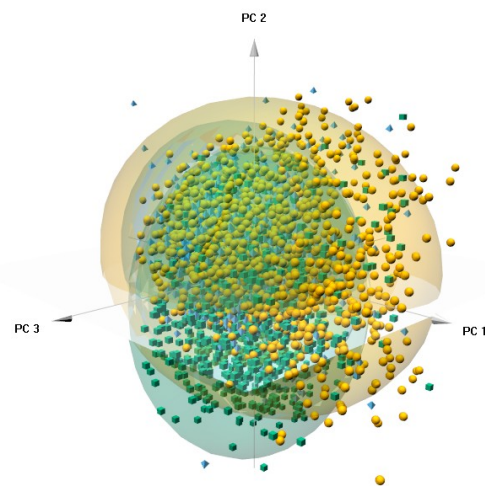
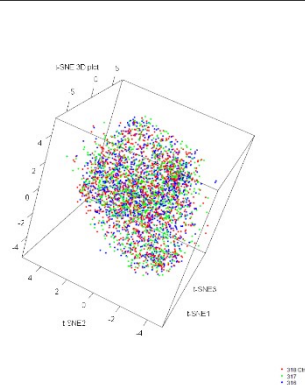
MitoTracker

Hoechst,
Concanavalin A,
MitoTracker

CELL PAINTING - HEATMAP

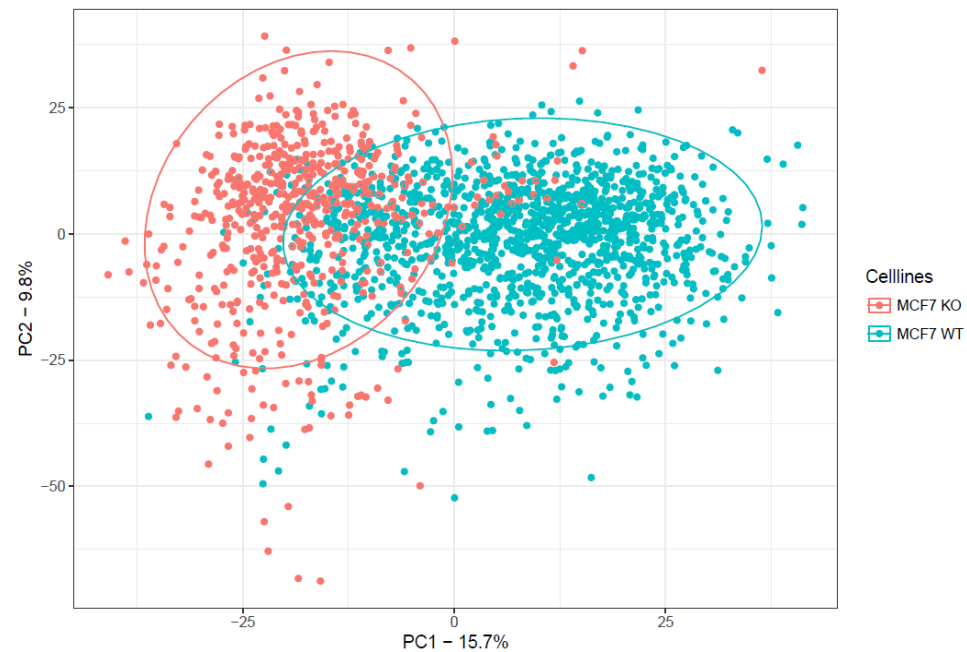
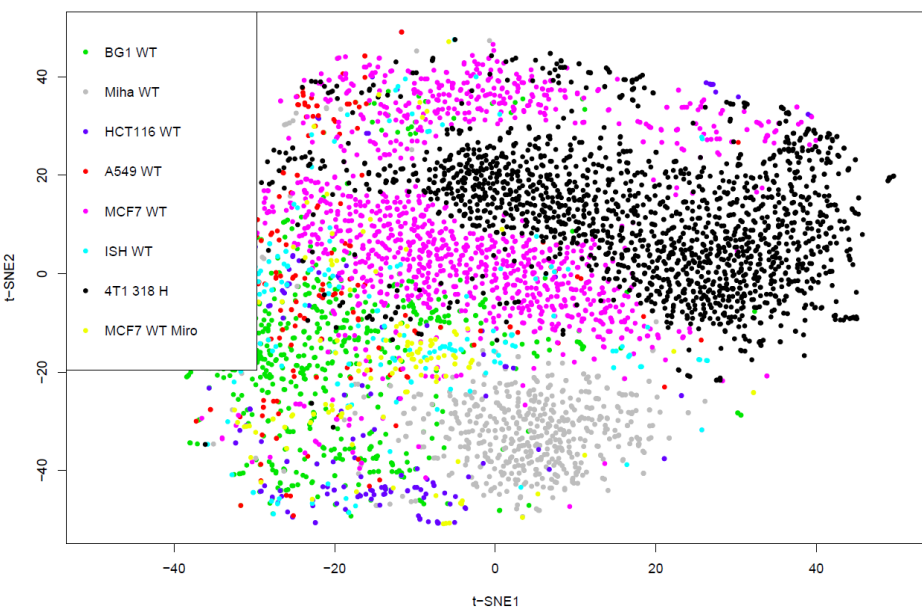


CELL PAINTING – PCA T-SNE



t-SNE plot

PCA Graph – points



IMAGEJ

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

FIJI

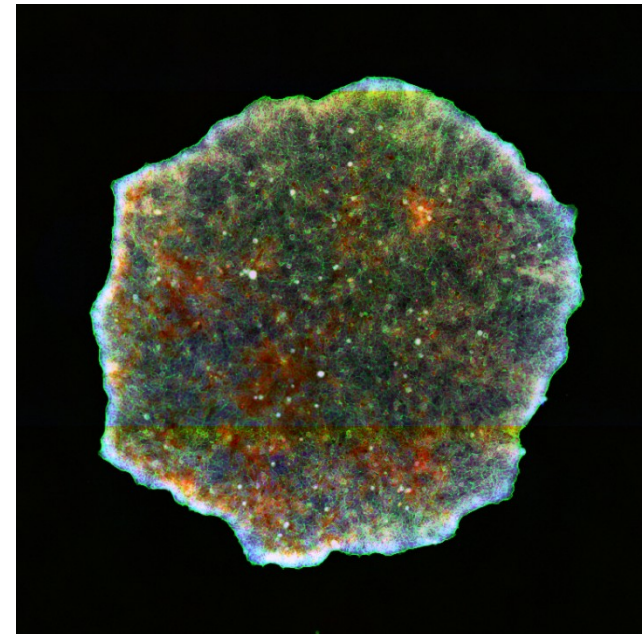
<http://fiji.sc/>

CELLPROFILER

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

ILASTIK

<http://ilastik.org/>



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7th Framework Programme
of the European Union

DĚKUJI ZA
POZORNOST

15.12.2020

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