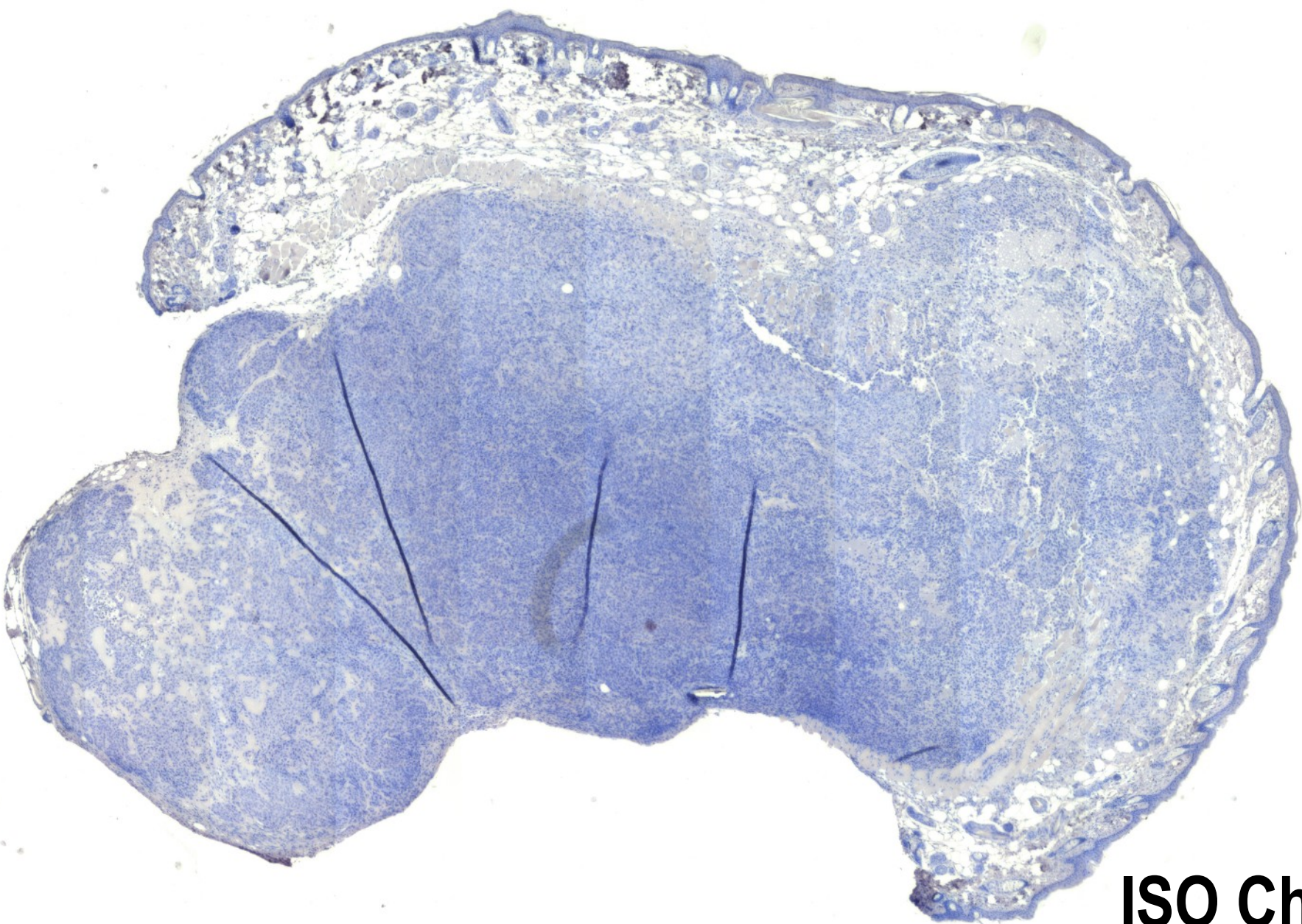


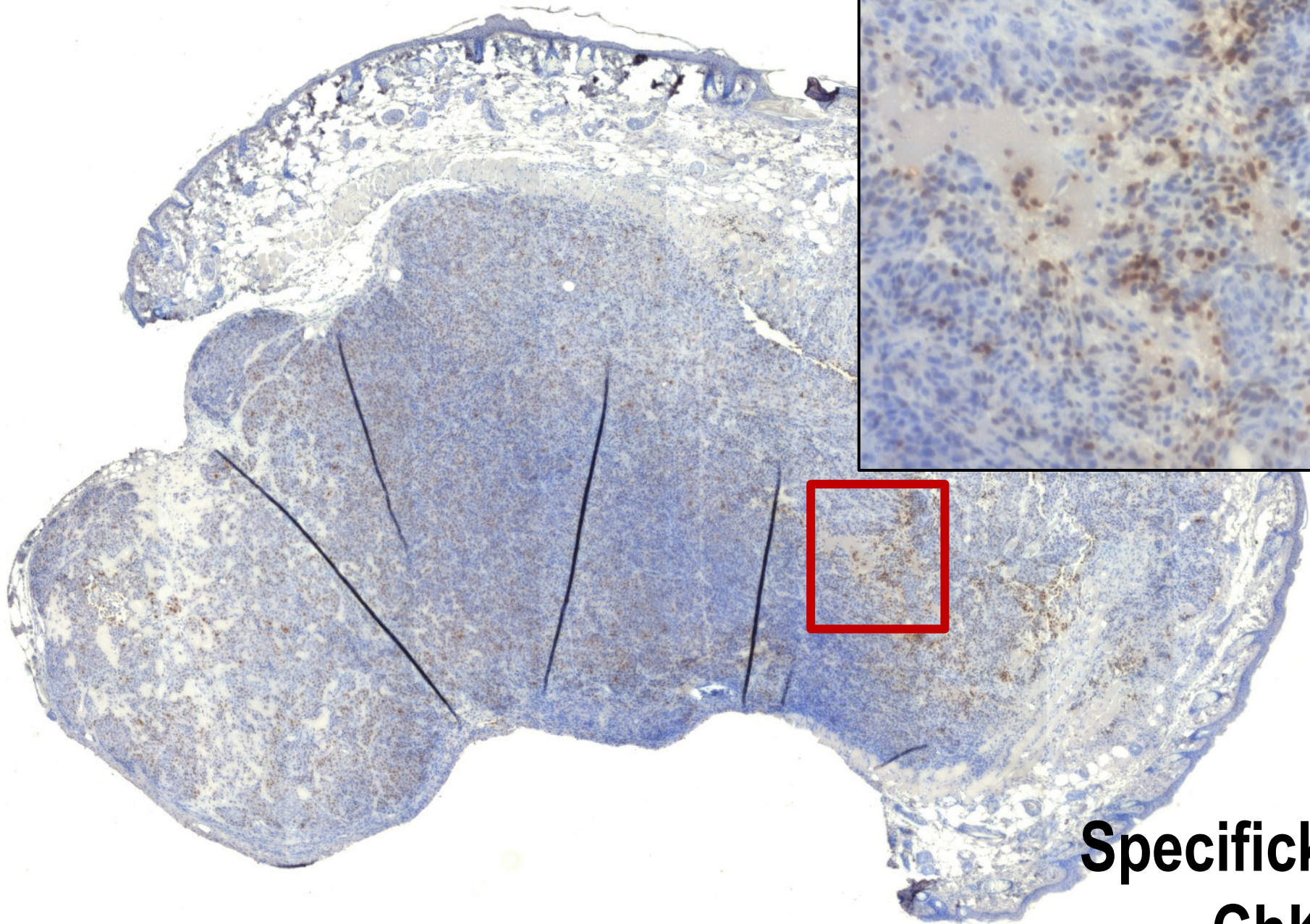
ZÁKLADNÍ PRINCIPY ANALÝZY OBRAZU

Příklady analýzy
obrazu

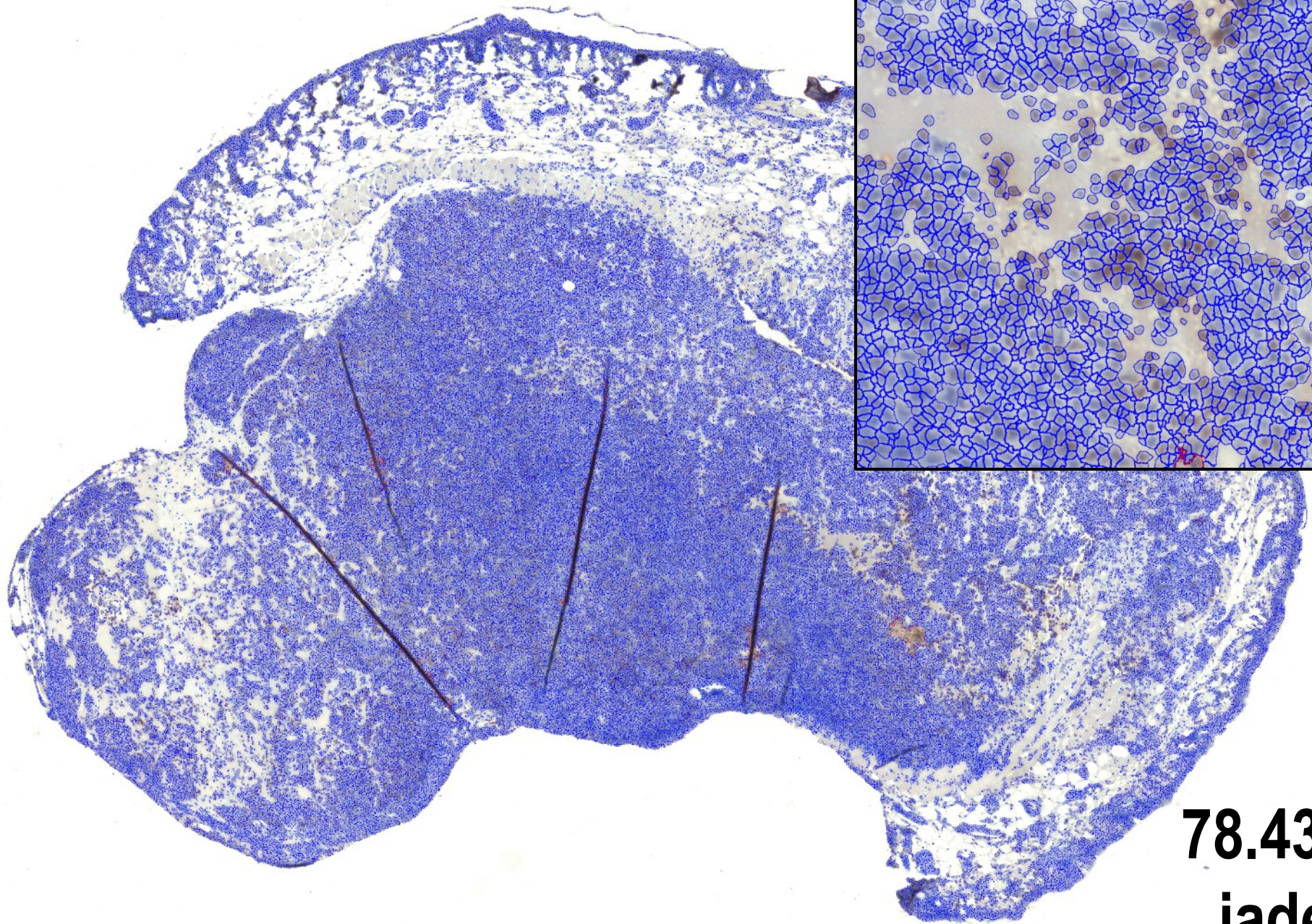
Radek Fedr



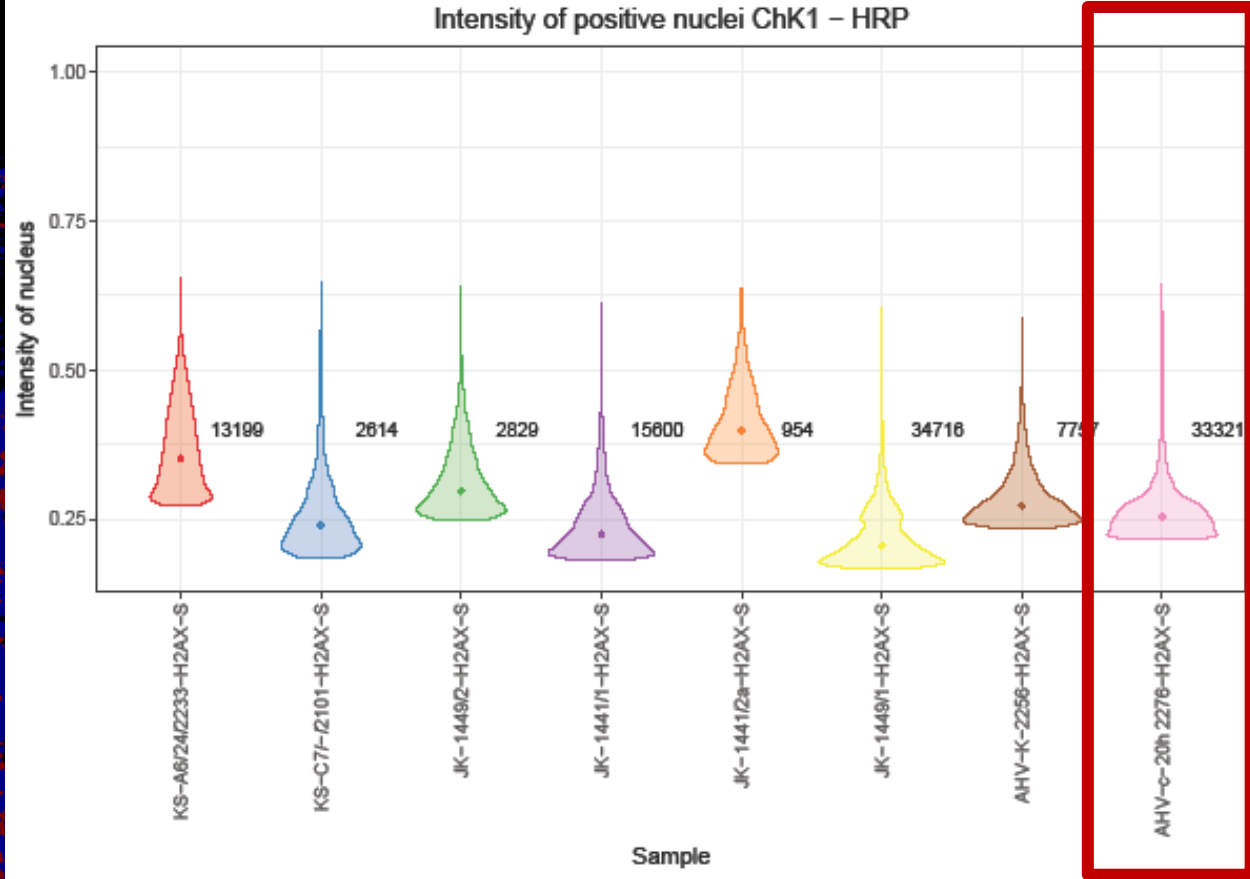
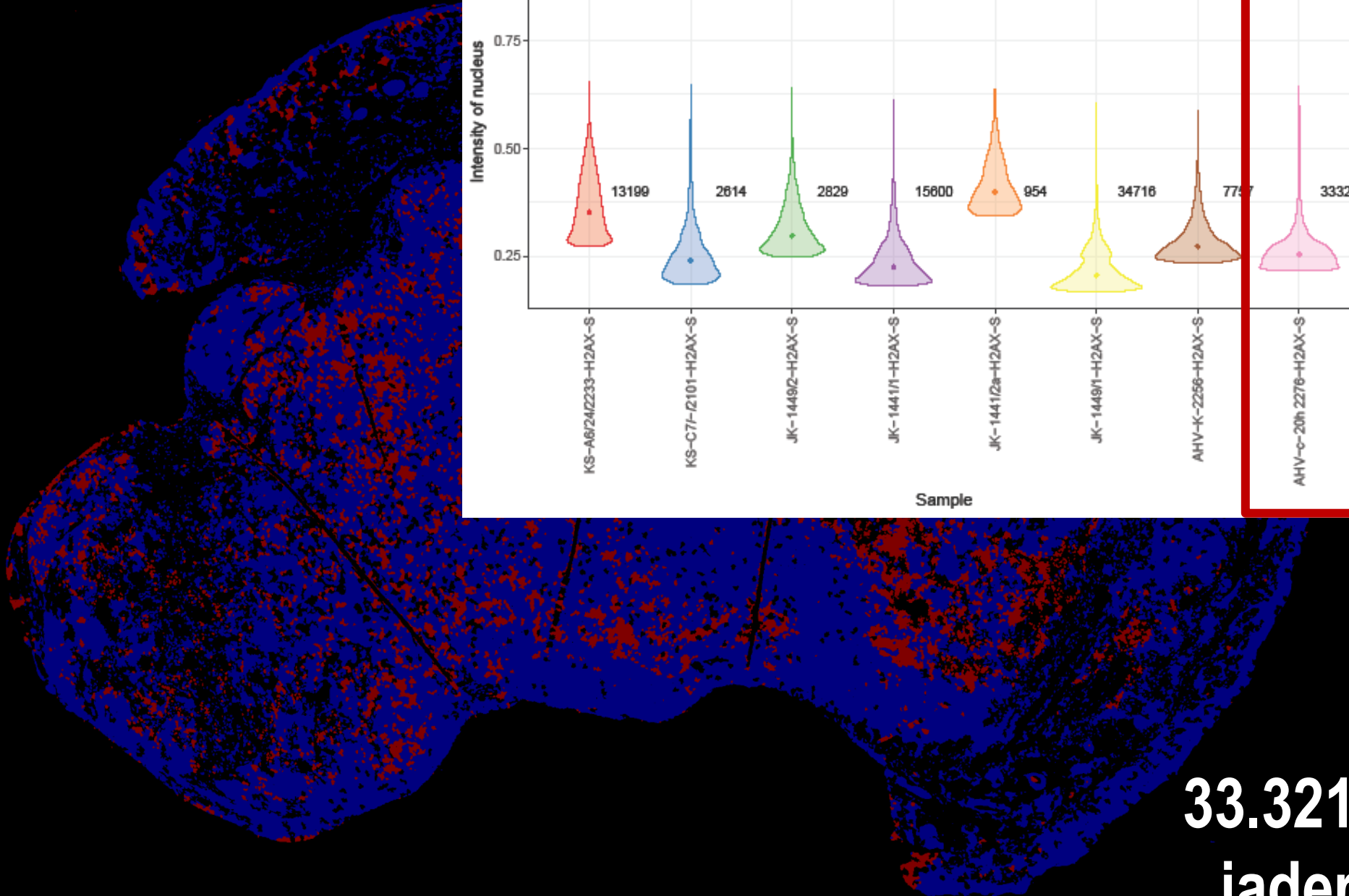
ISO ChK1



**Specifický
ChK1**



78.436
jader



33.321
jader

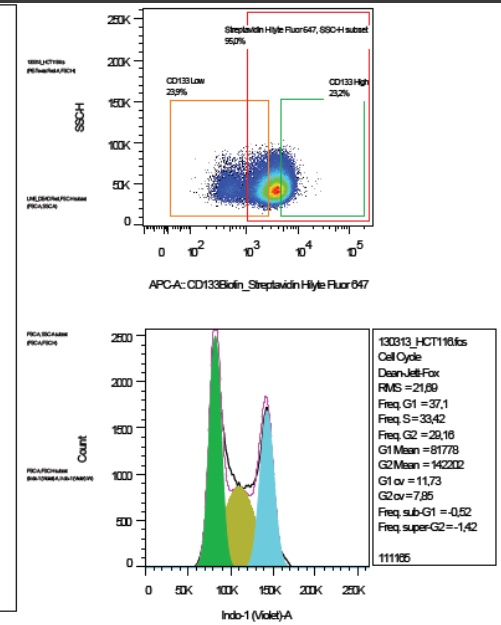
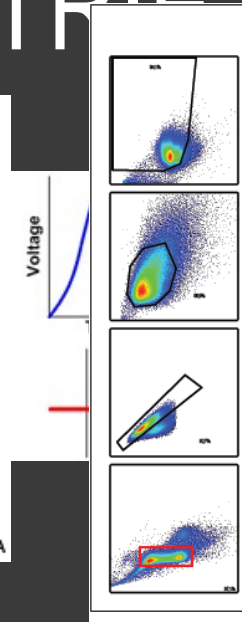
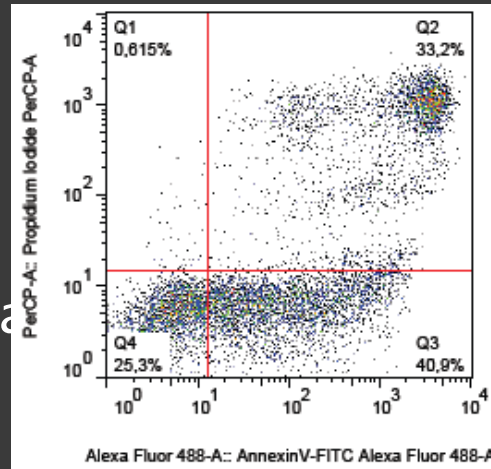
ANALÝZA TKÁNÍ

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



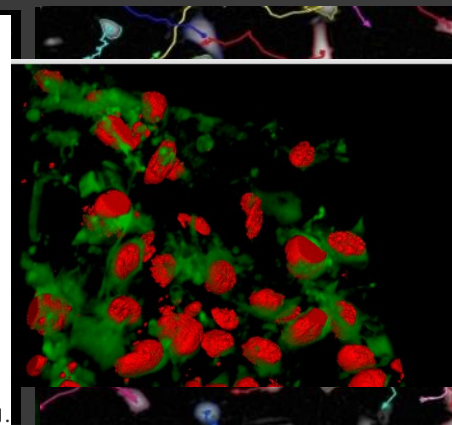
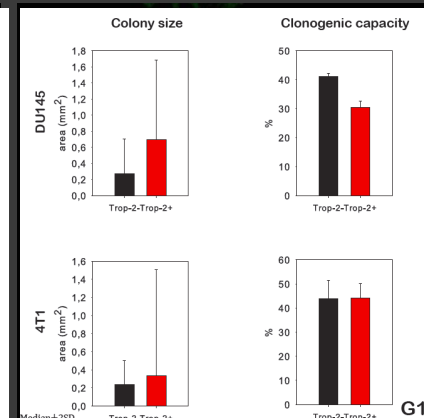
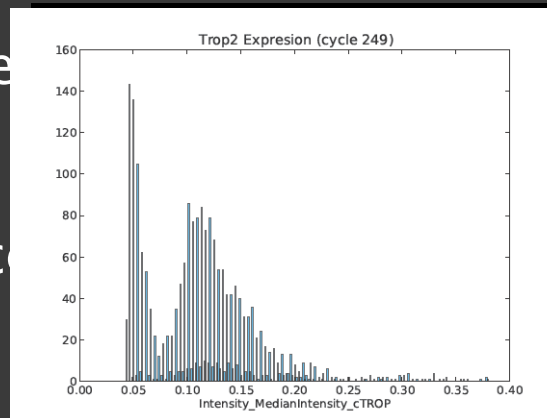
PRŮTOKOVÁ CYTOMETRIE

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



OBRAZOVÁ CYTOMETRIE

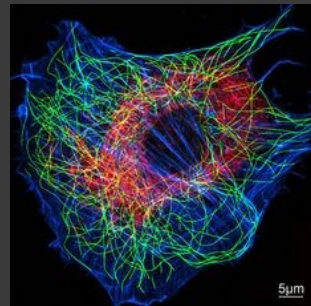
- Pixel
- Intenzita - pixel
- Distribuce
- Obraz, Projekce
- Graf, Model
- Tracking/Sledování



SROVNÁNÍ

Mikroskopie

Ukázka



Vzorek

2D, 3D

Rozlišení

100nm

Fluorescenční parametry

1-5

Rychlost

100 buněk/s

Data

100GB

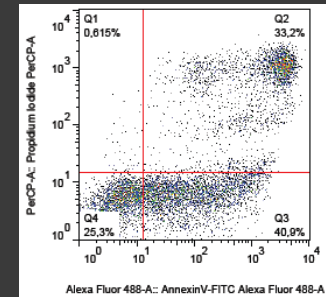
Kvantifikace

Přesná

Počet buněk

10^1 - 10^6

Průtoková cytometrie



Suspenze

1 částice/1µm

10+

10000 buněk/s

100MB

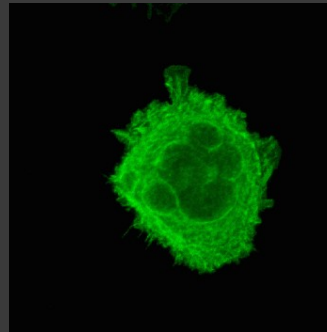
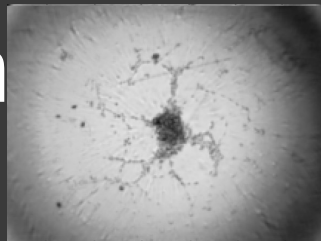
Přesná

10^4 - 10^7

PŘÍPRAVA A SNÍMÁNÍ

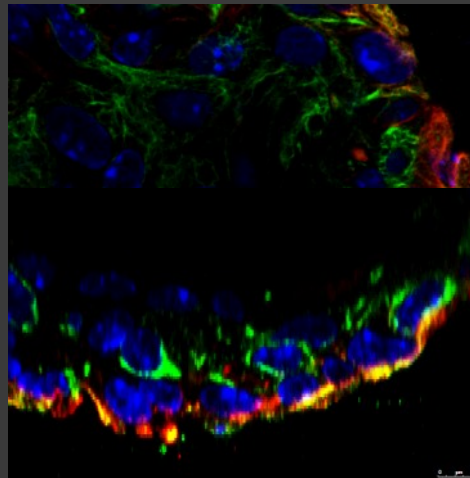
Experiment

- živé
- fixované
- 2D
- 3D



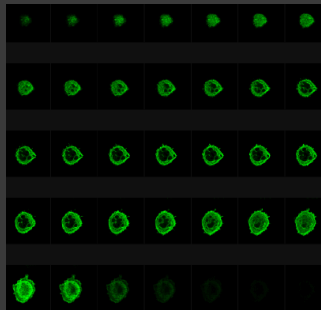
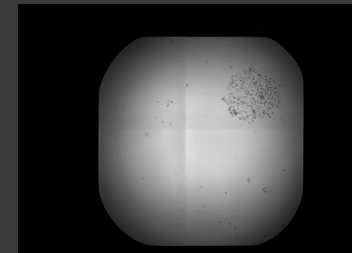
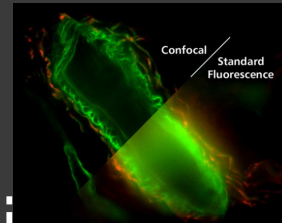
Vzorek

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

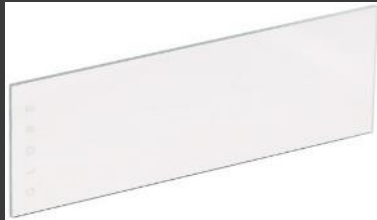


Mikroskopie

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

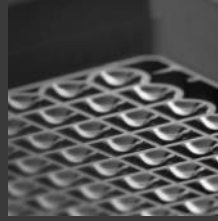


PŘÍPRAVA A SNÍMÁNÍ



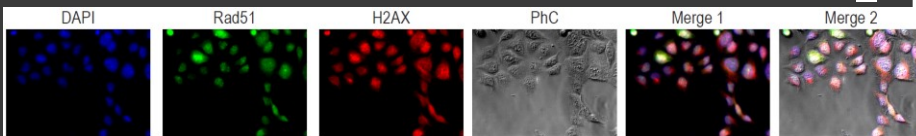
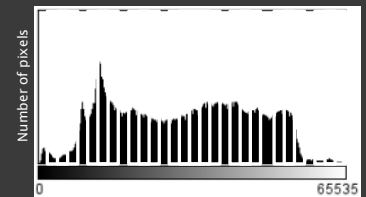
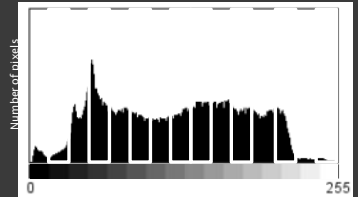
Plast

- podložní sklo
- ztenčené dno
- více-jamkové desky



Rozlišení

- objektiv
- bitová hloubka
- počet barev



SNÍMÁNÍ



- Příprava

- Metodické

- Zpracování

vzorku

- Popisné

- Xylografické

“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

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

ImageJ:
Analyze →
Histogram
Image → Type

HISTOGRAM – BITOVÁ HLOUBKA

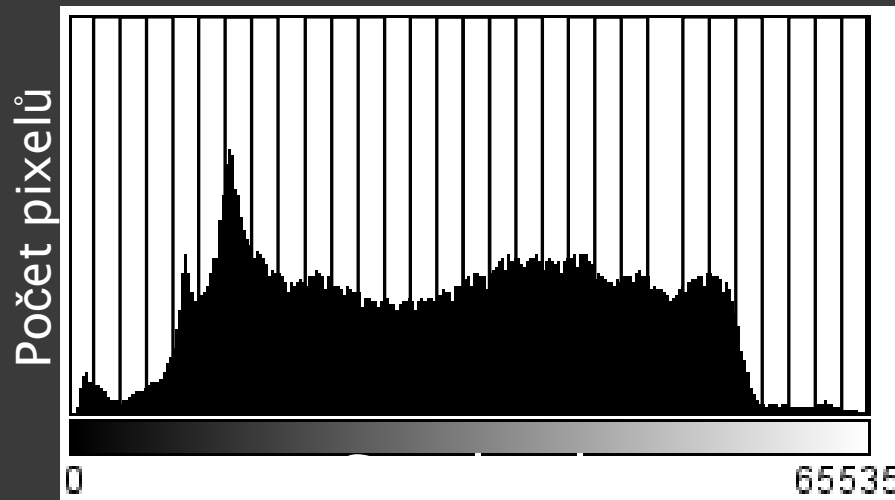
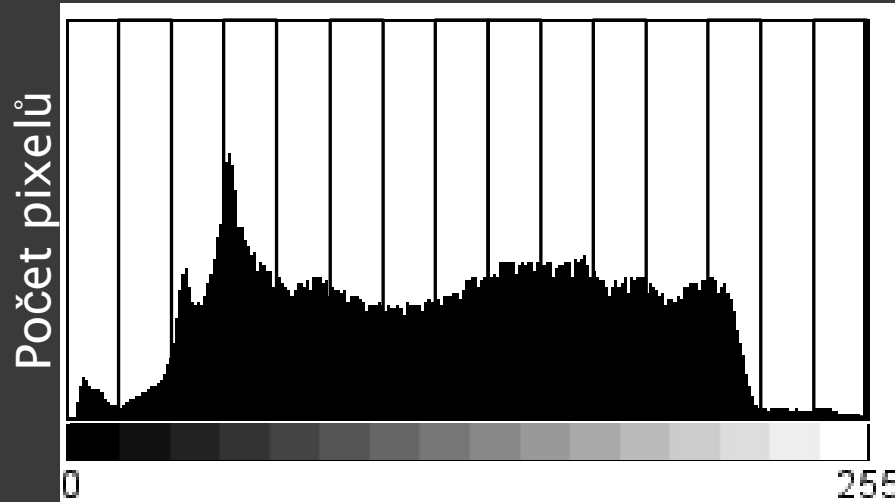
Originální obrázek

Histogram

Bitová
hloubka

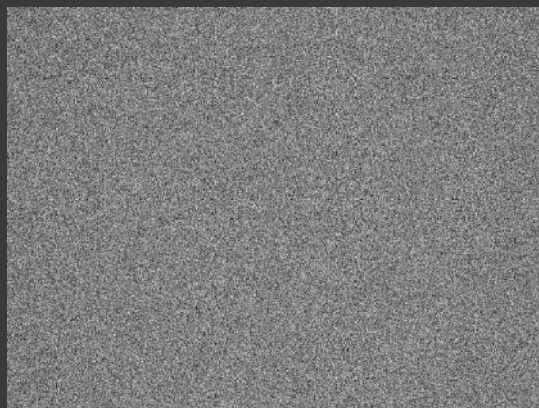
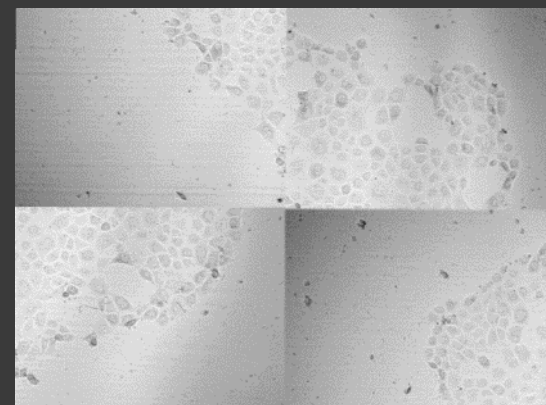
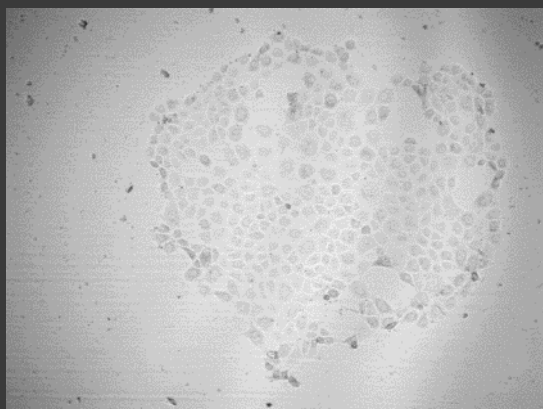
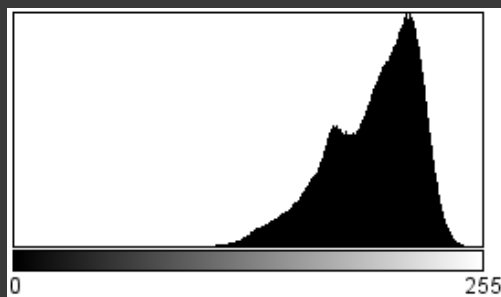
8 bit

16 bit



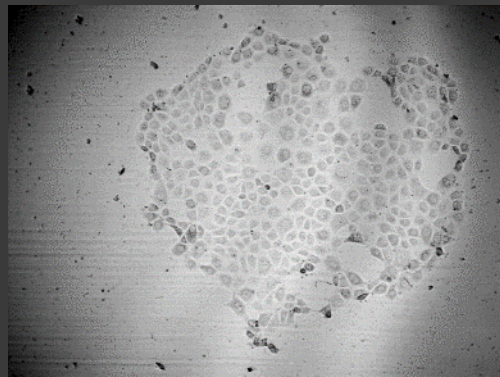
HISTOGRAM – REKONSTRUKCE OBRAZU

Histogram

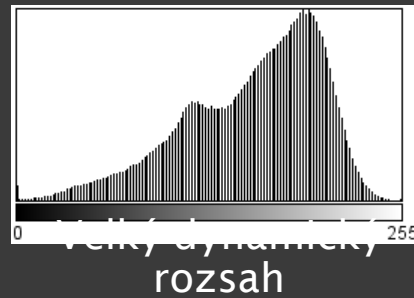


ImageJ:
Image → Adjust
→
Brightness/Contrast

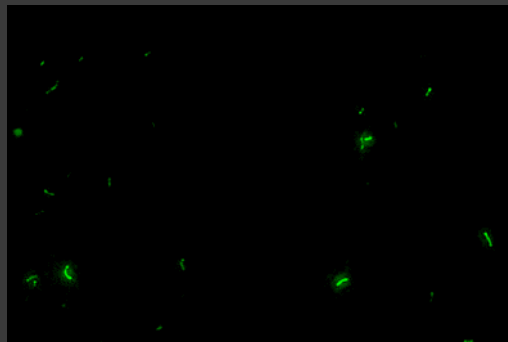
HISTOGRAM – DYNAMICKÝ ROZSAH



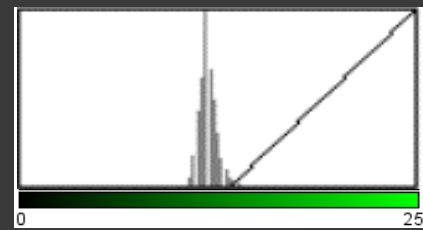
Histogram
s Originál



Malá expozice



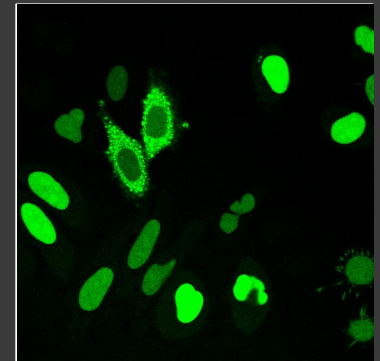
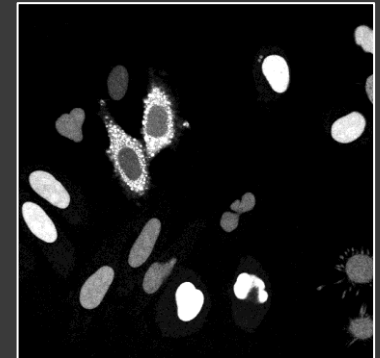
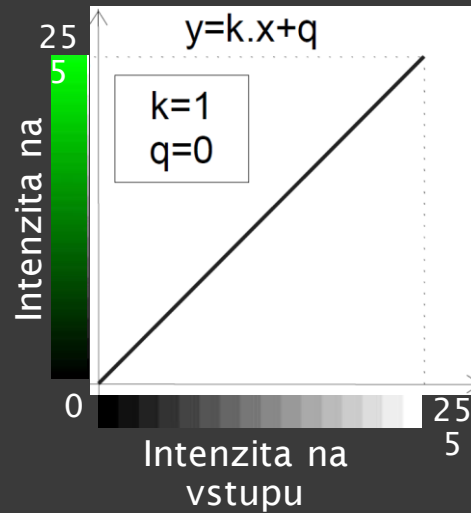
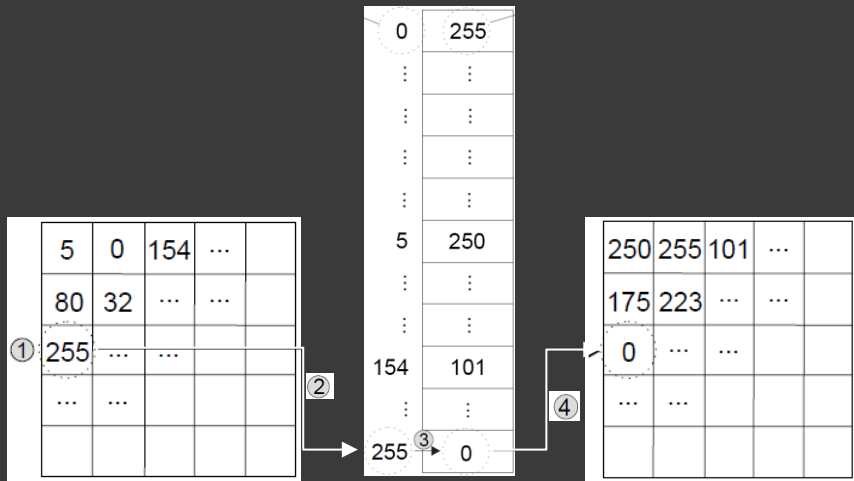
Odstranění pozadí



ImageJ:
 Image →
 Lookup Tables
 → Invert
 LUT/Green

HISTOGRAM & LUT – LOOKUP TABLE

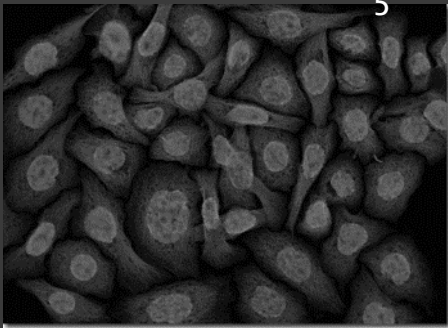
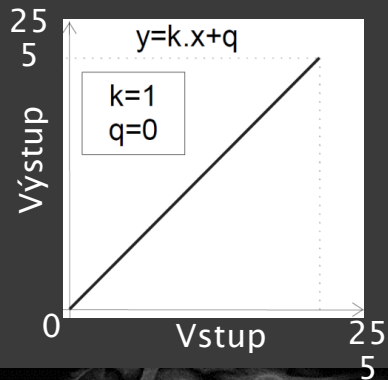
Jak LUT funguje



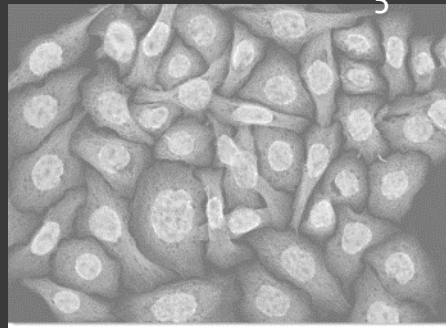
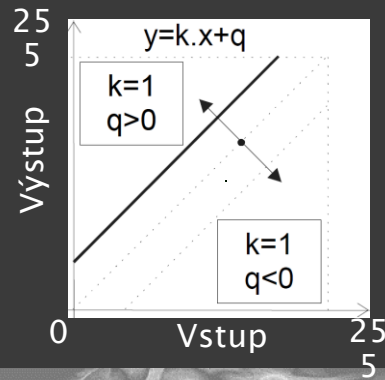
ImageJ:
Image → Adjust
→
Brightness/Contrast

HISTOGRAM & LUT – PŘÍKLADY

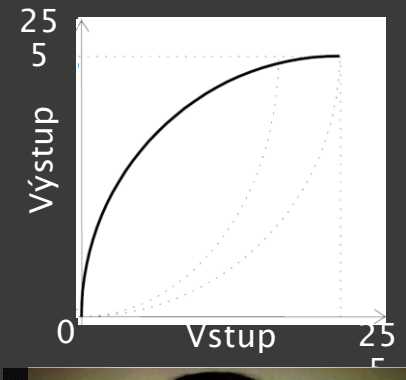
Žádná změna



Jas

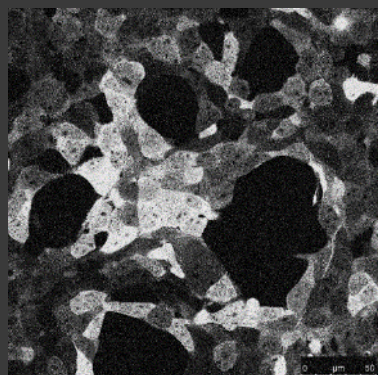


Kontrast

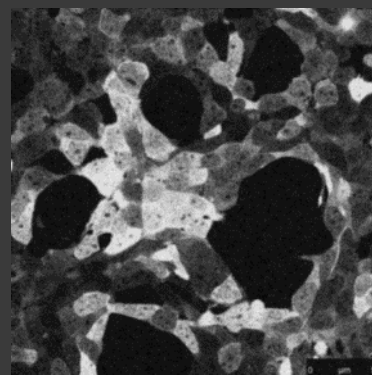


ImageJ:
Process → Filters
→ Gaussian
Blur/Median

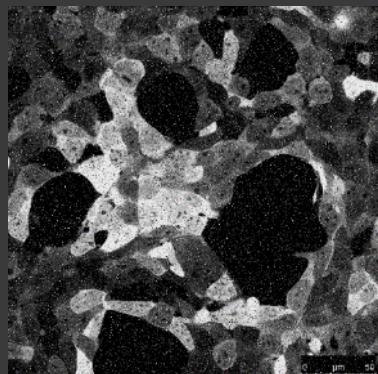
HISTOGRAM - ŠUM



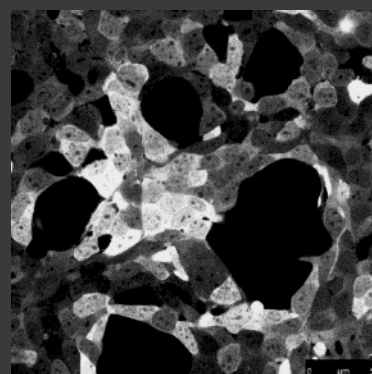
Gaussovský



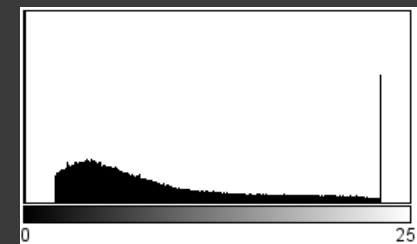
Blur filtering



Sůl & Pepř



Median filtering



DATOVÉ FORMÁTY

Rastrové obrazy

Tvar reprezentovaný maticí pixelů

Pixelace

Fotorealistické obrazy

Typické formáty: JPEG, GIF, TIFF, PNG

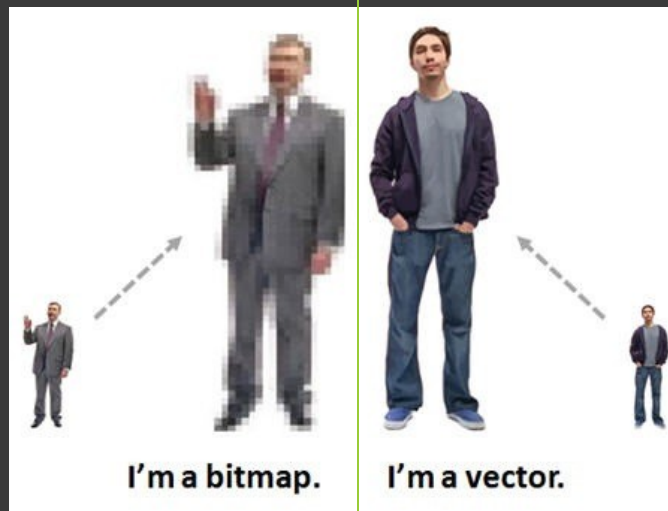
Vektorové obrazy

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
prost

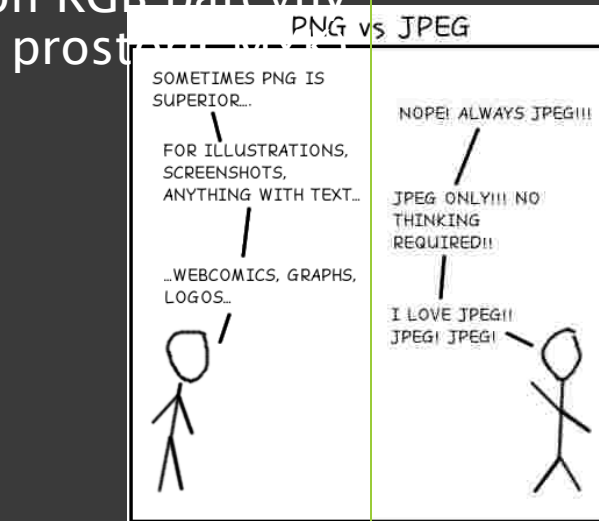
Joint Photographic Expert Group (JPEG)

Fotky a realistické obrazy

Ztrátová komprese

Jemné, pozvolné přechody

Nepodporuje transparentnost



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



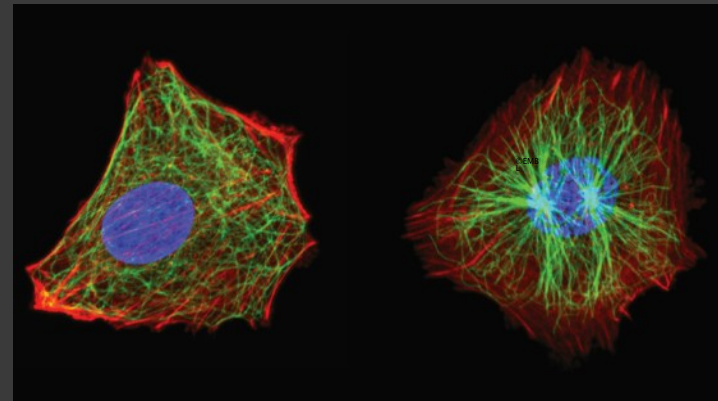
Tagged Image File Format (TIFF)

Fotky, grafika

Možnost bezeztrátové komprese

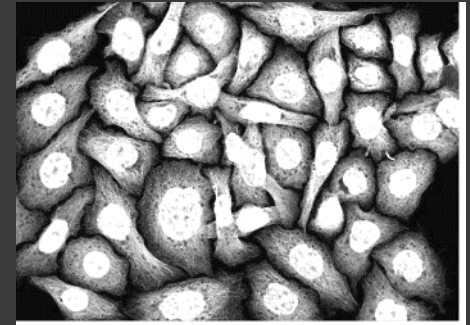
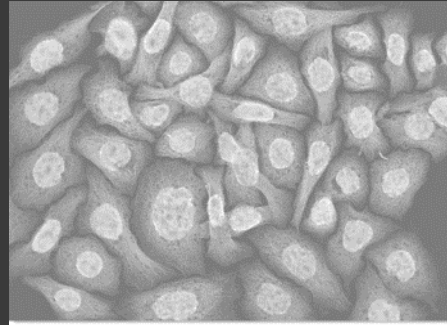
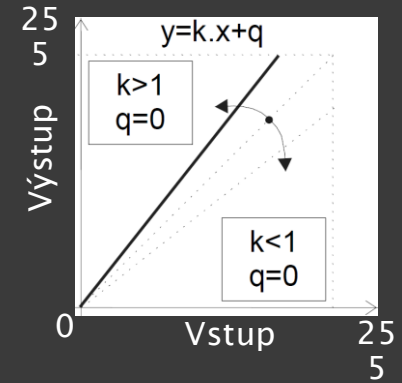
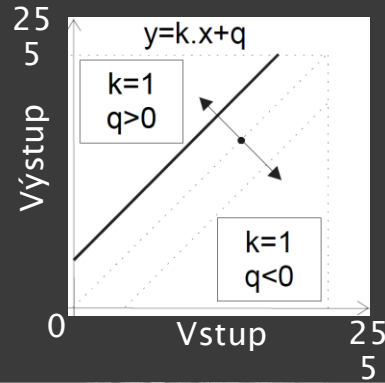
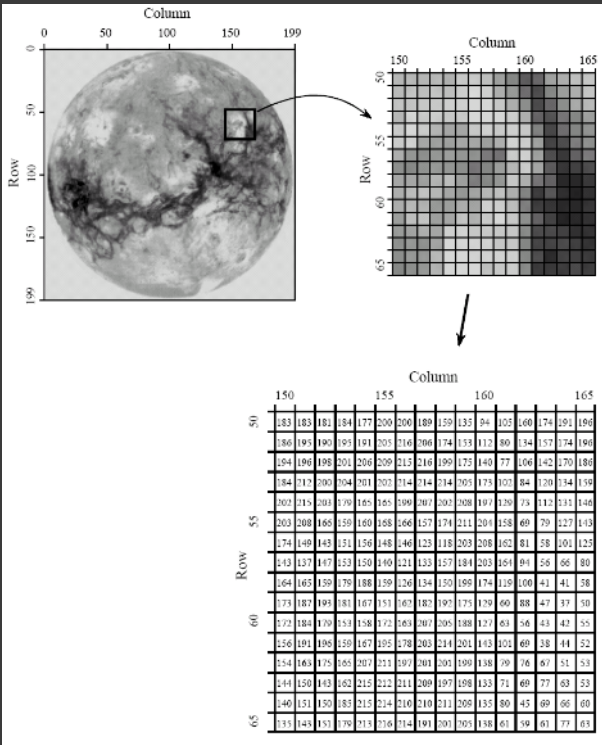
Profesionální, široce podporovaný

Různé barevné prostory, jakékoli
rozlišení a barvy



ZPRACOVÁNÍ – BODOVÉ TRANSFORMACE

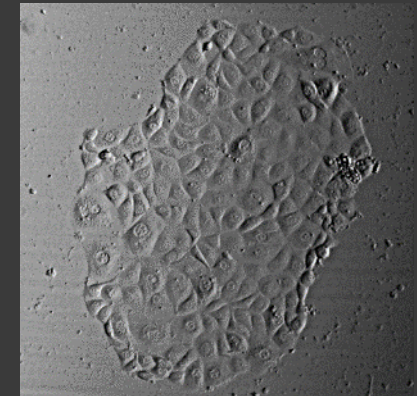
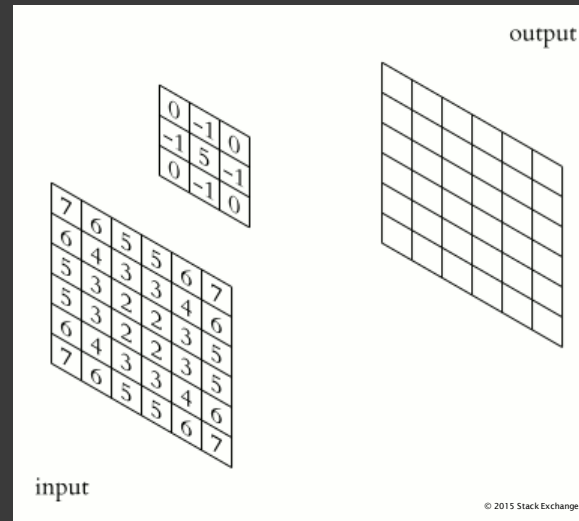
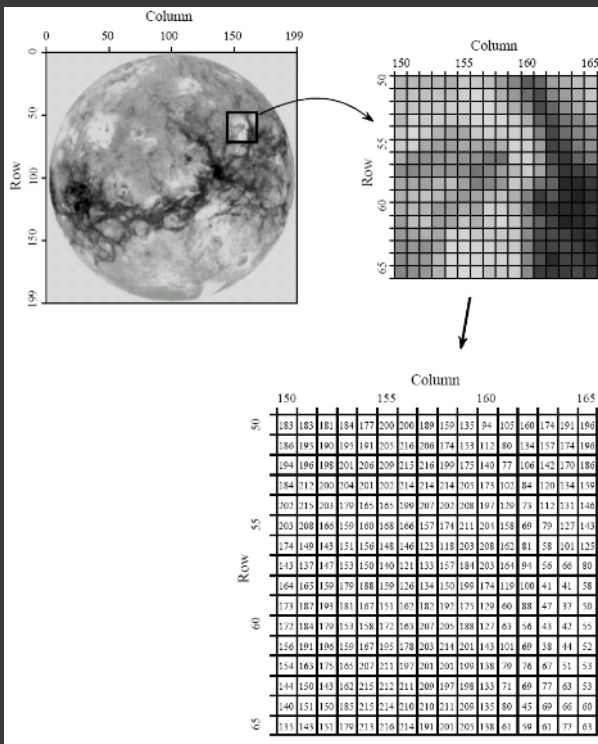
ImageJ:
Image → Adjust
→
Brightness/Contrast



✖ 30

ImageJ:
Process →
Sharpen/Filters
→ Unsharp Mask

FILTROVÁNÍ – LOKÁLNÍ ZMĚNY



ImageJ:

Process →

Sharpen/Smooth/

Find Edges

Plugins → Parallel

Iterative

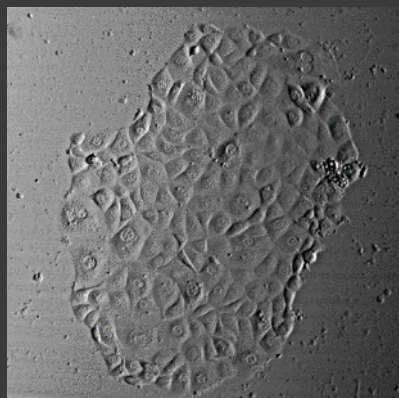
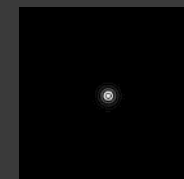
Deconvolution

FILTROVÁNÍ – PŘÍKLADY

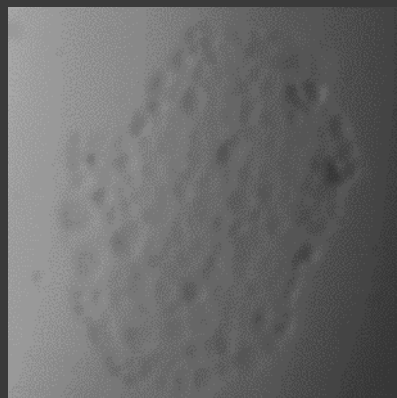
0	0	0	0	0
0	0	-1	0	0
0	-1	5	-1	0
0	0	-1	0	0
0	0	0	0	0

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

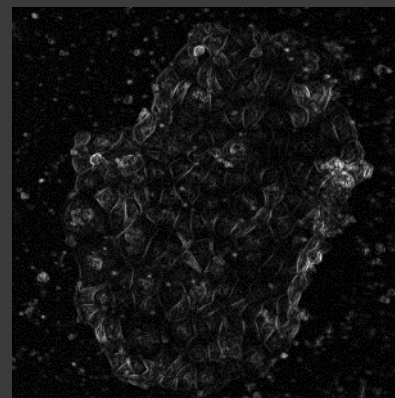
	0	1	0	
	1	-4	1	
	0	1	0	



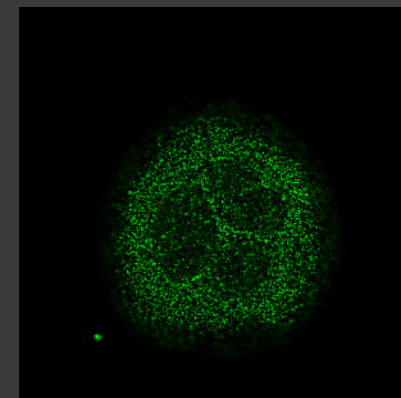
doostření



rozmazání



detekce hran

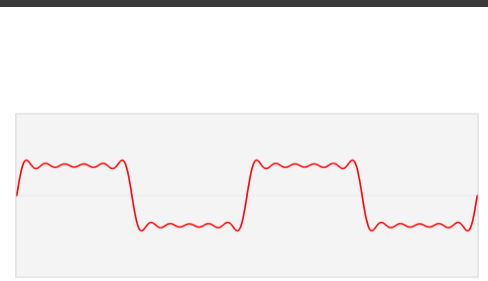
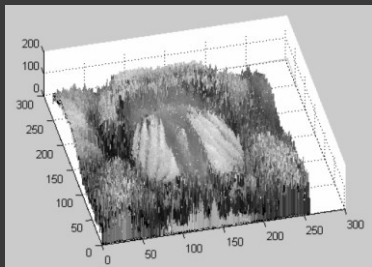
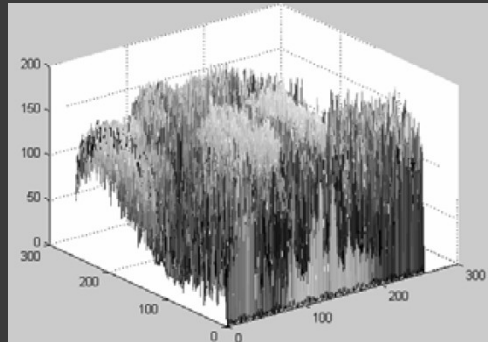


dekonvoluce

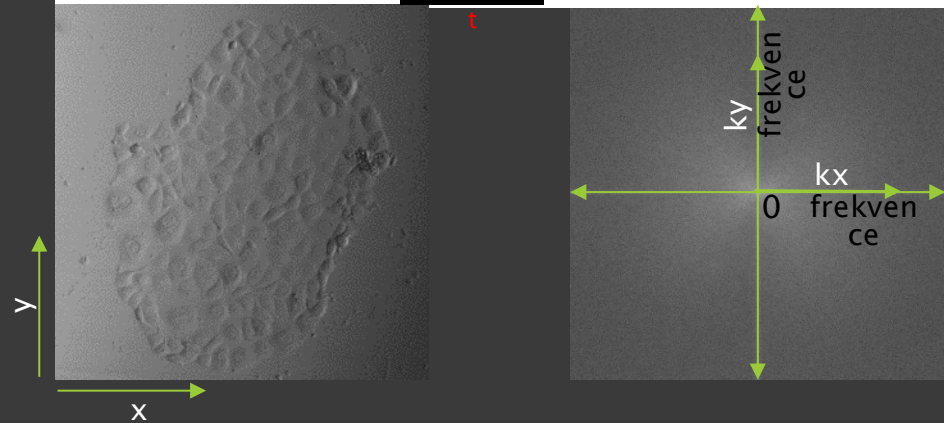
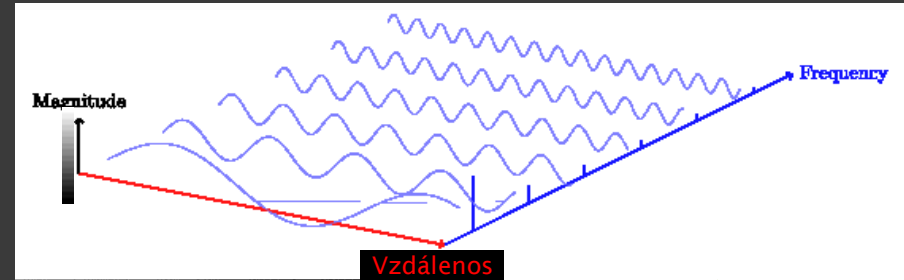
ImageJ:
Process → FFT →
FFT

FILTROVÁNÍ – GLOBÁLNÍ TRANSFORMACE

Fast Fourier Transformation

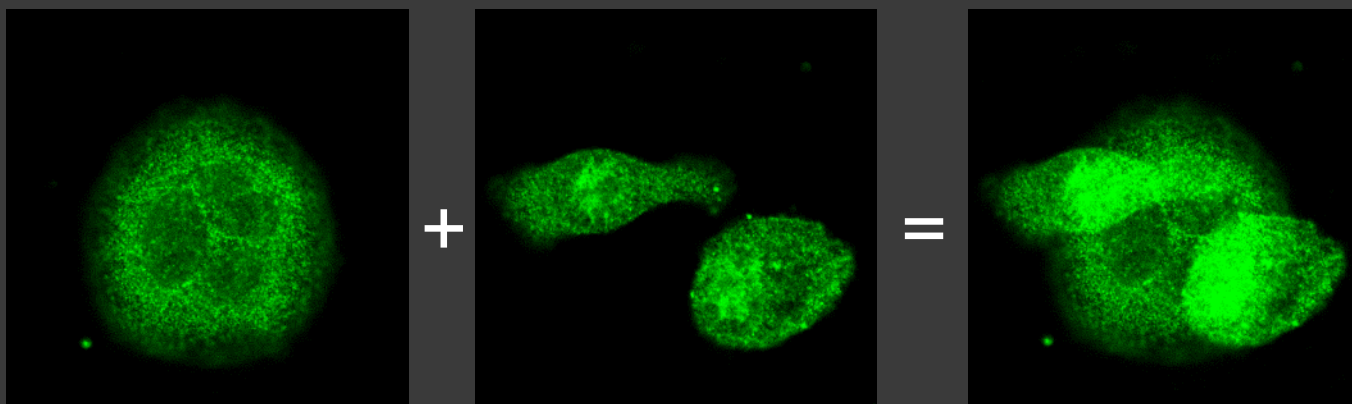


Konvoluce = Násobení

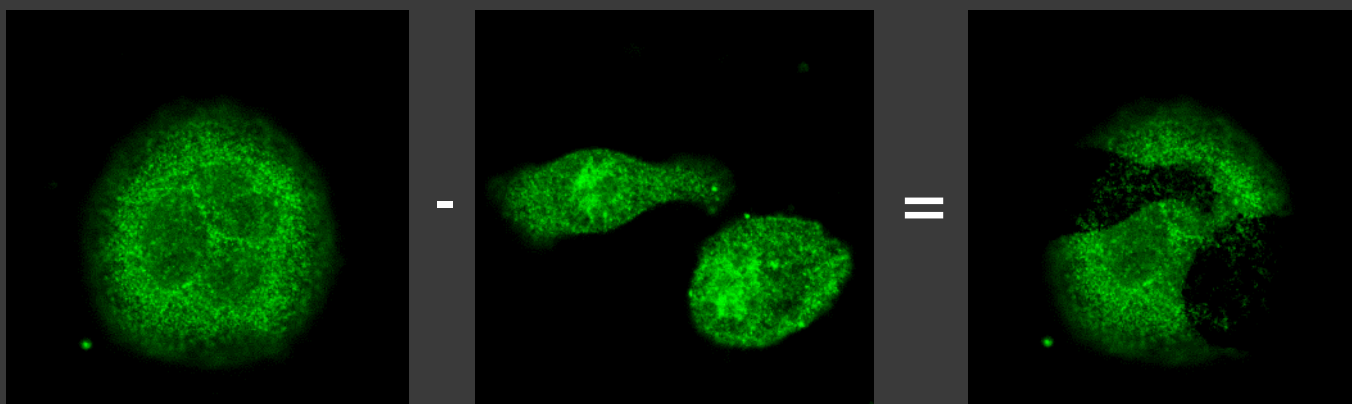


OBRAZOVÁ ARITMETIKA

Sčítání

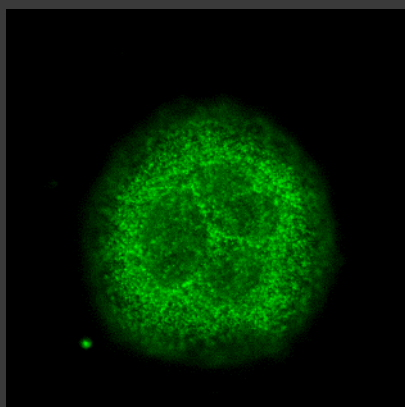


Odčítání

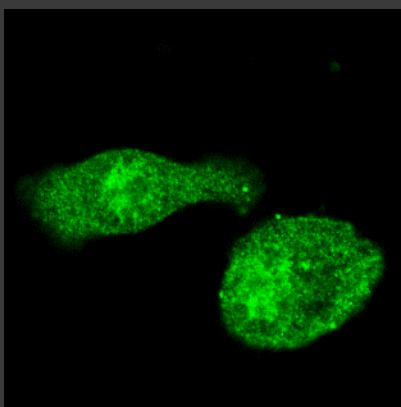


OBRAZOVÁ ARITMETIKA

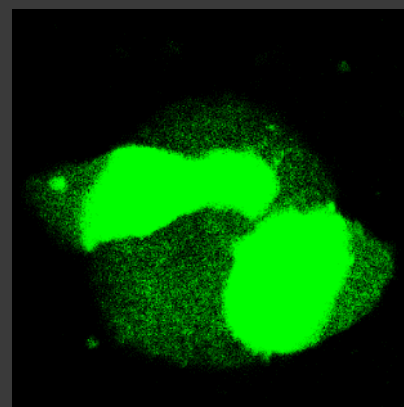
Násobení



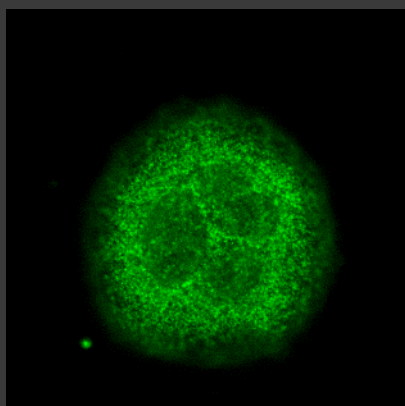
x



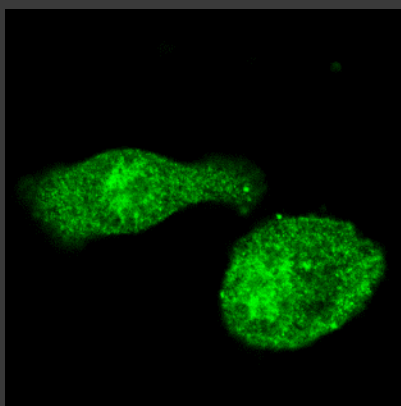
=



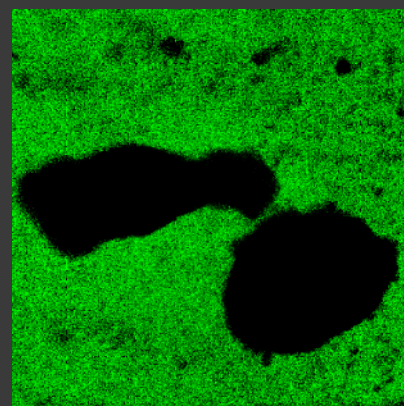
Dělení



/

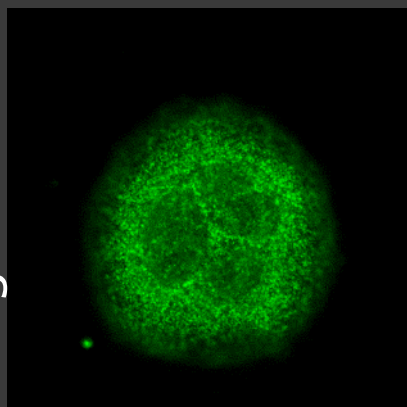


=

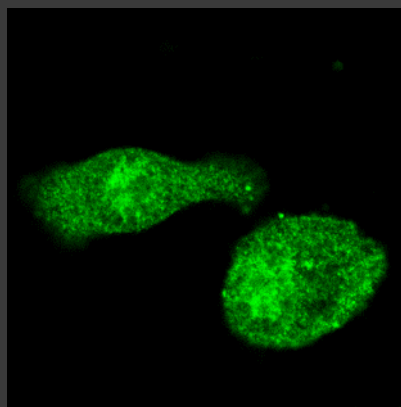


OBRAZOVÁ ARITMETIKA

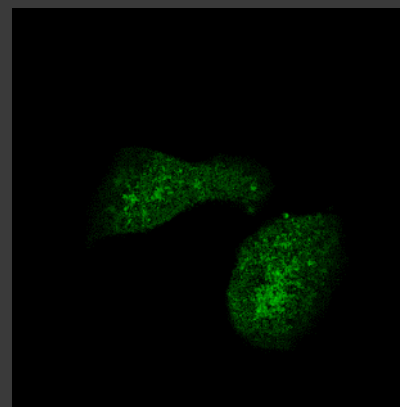
Logické AND



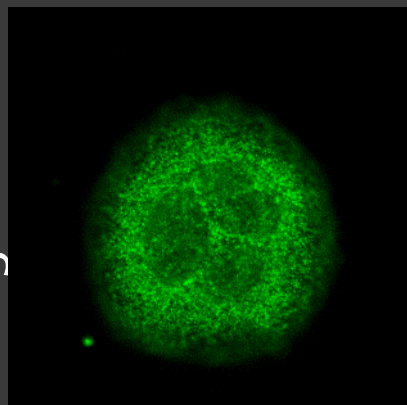
AND



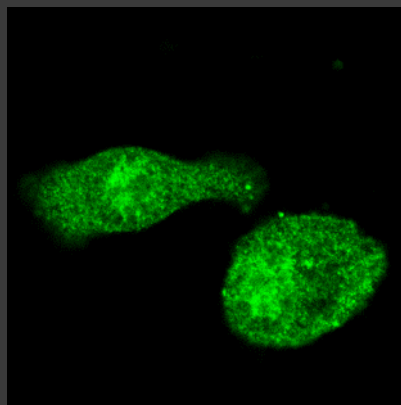
==



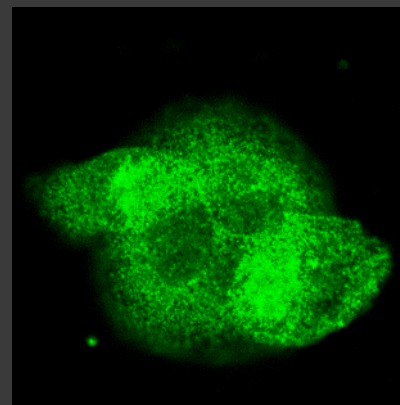
Logické OR



OR



==



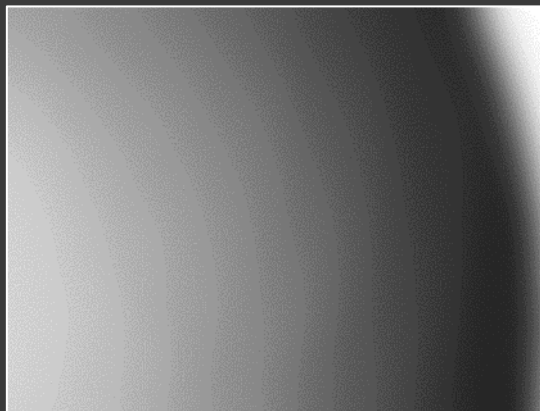
OBRAZOVÁ ARITMETIKA – PŘÍKLADY

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

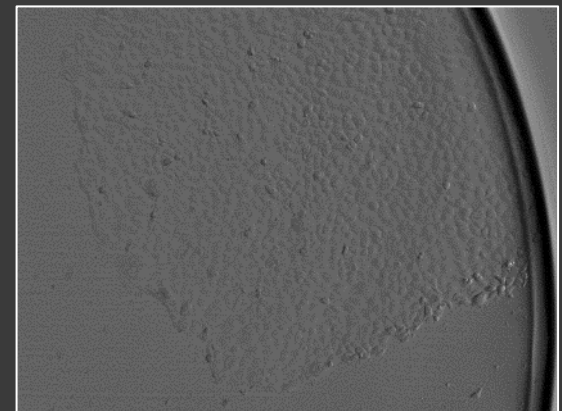
Flat Field korekce



/



=

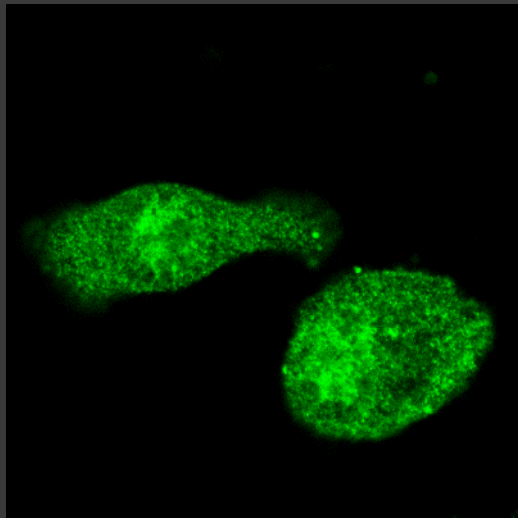


Gaussian blur

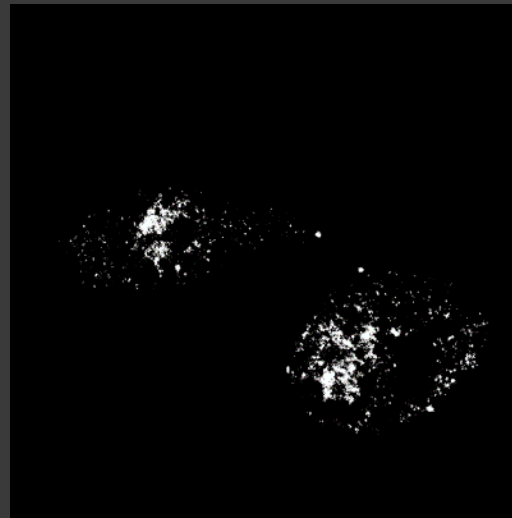
OBRAZOVÁ ARITMETIKA – PŘÍKLADY

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

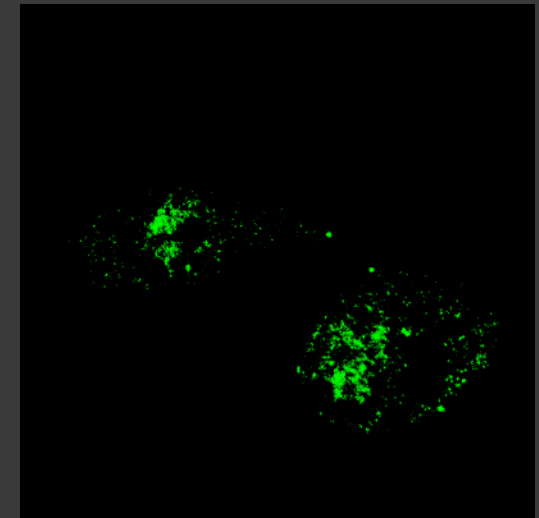
Segmentace



AND



=

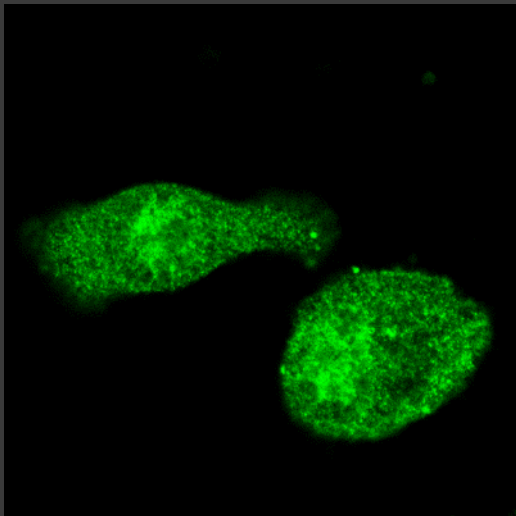


Maska

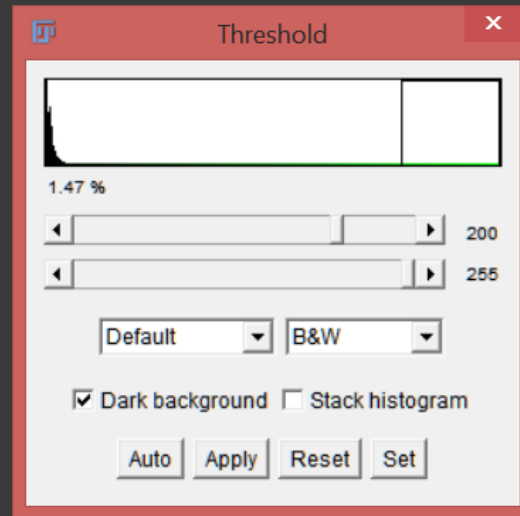
ImageJ:
Image → Adjust
→ Threshold

SEGMENTACE – MANUÁLNÍ

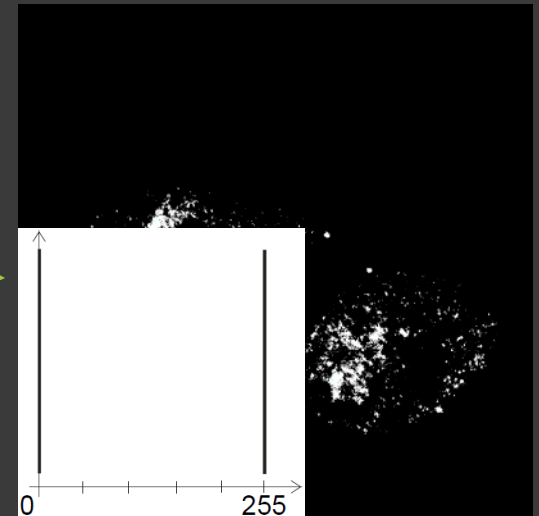
Originální obraz



Prahování



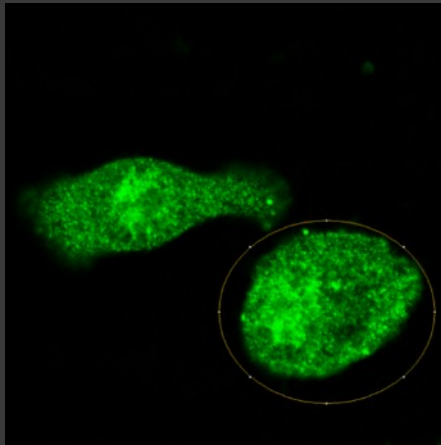
Maska



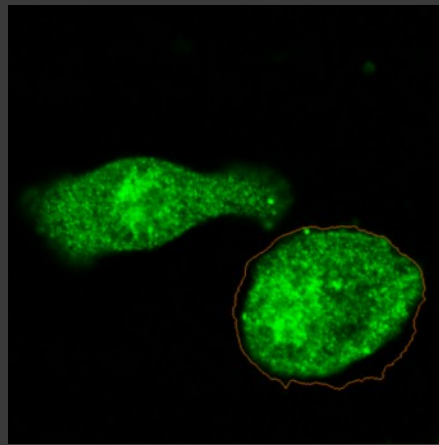
ImageJ:
Plugins →
Segmentation →
Level Sets

SEGMENTACE – POLOAUTOMATICKÁ

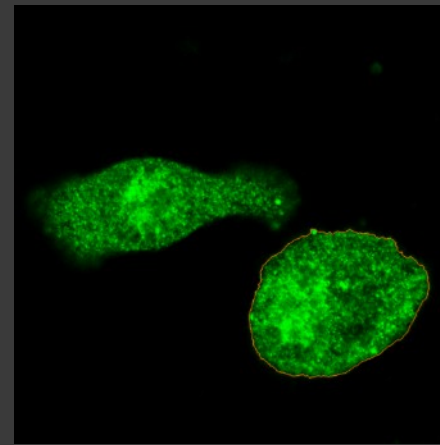
Start - ROI



Level sets



Konec



Maska



ImageJ:
Plugins →
Segmentation →
Level Sets

SEGMENTACE – POLOAUTOMATICKÁ



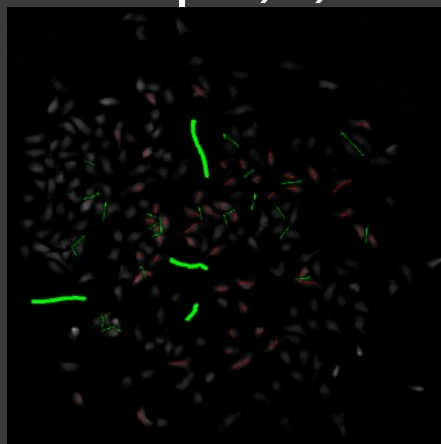
SEGMENTACE – STROJOVÉ UČENÍ

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

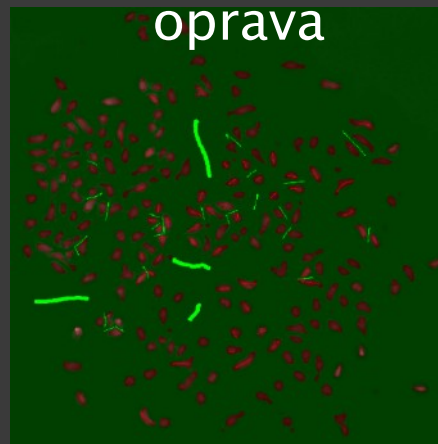
Originální
obraz



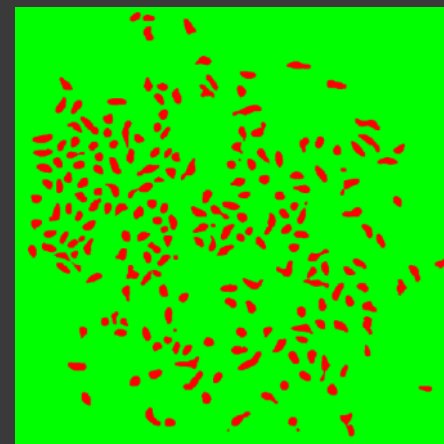
Učení



Kontrola a
oprava



Maska



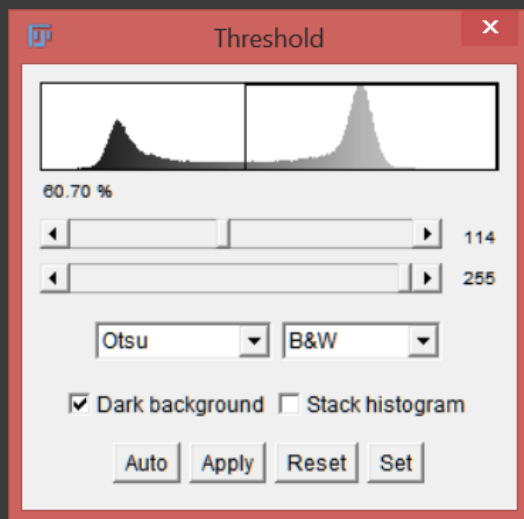
ImageJ:
Image → Adjust
→ Threshold →
Otsu

SEGMENTACE – AUTOMATIZOVANÁ

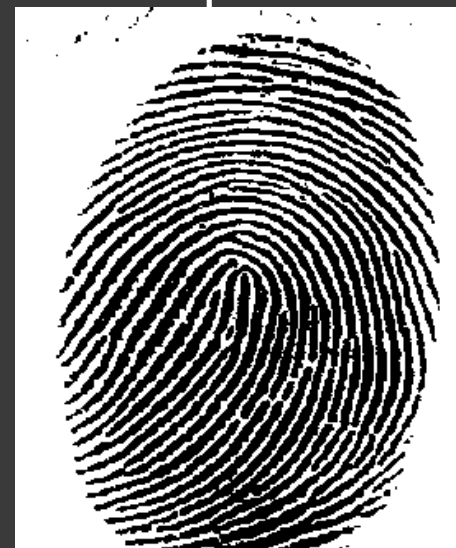
Originální



Otsu - Klastrování



Segmentovaný

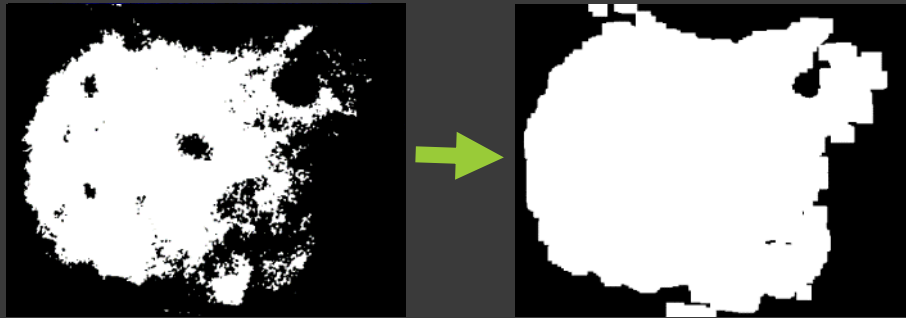


Intra-class variance
minimal

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

ZMĚNA MORFOLOGIE MASKY

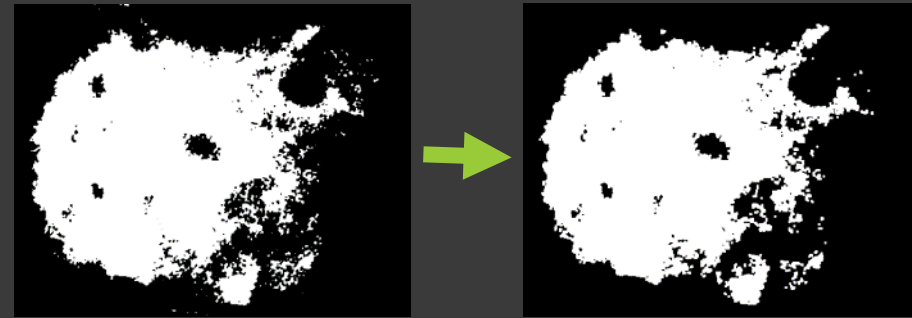
Roztažení



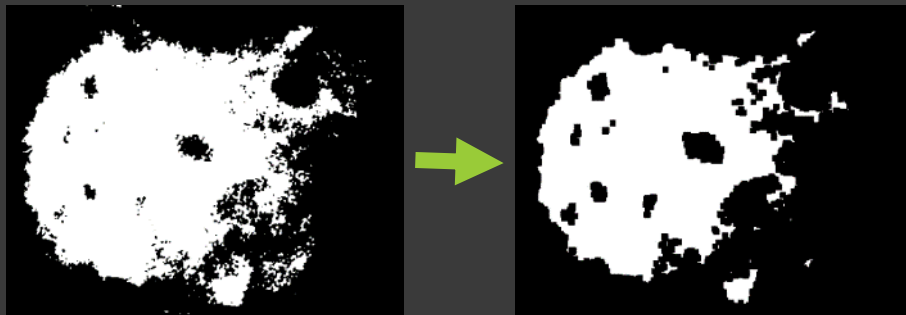
+ Structuring element



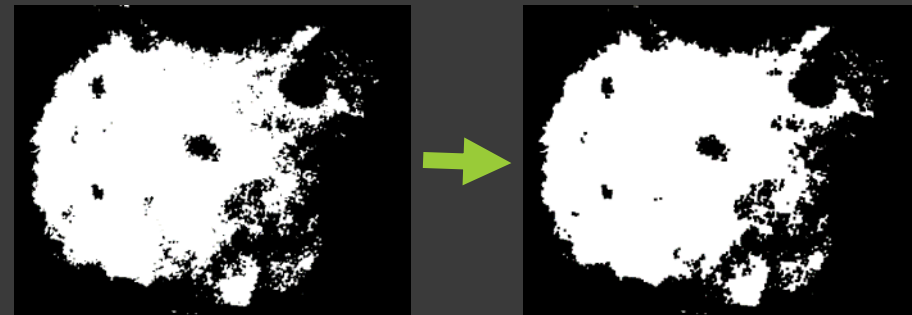
Otevření



Eroze



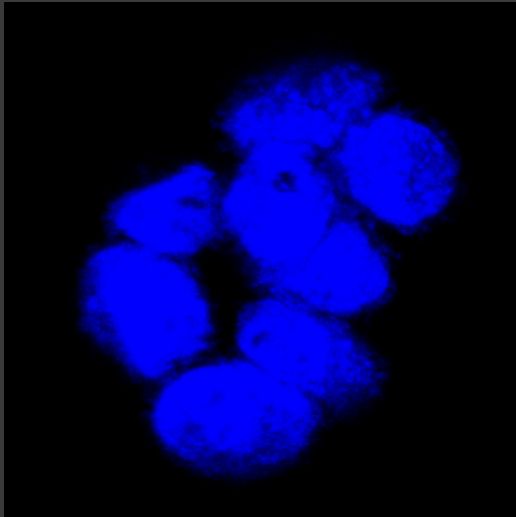
Zavření



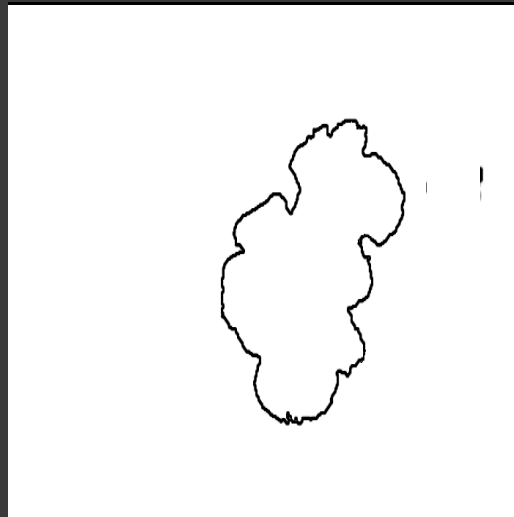
ImageJ:
Process → Binary
→ Make Binary
Process → Binary
→ Watershed

ZMĚNA MORFOLOGIE MASKY

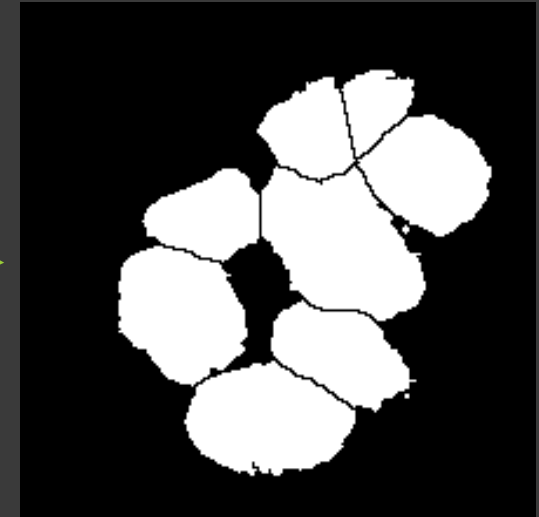
Originální obrazy



Prahování



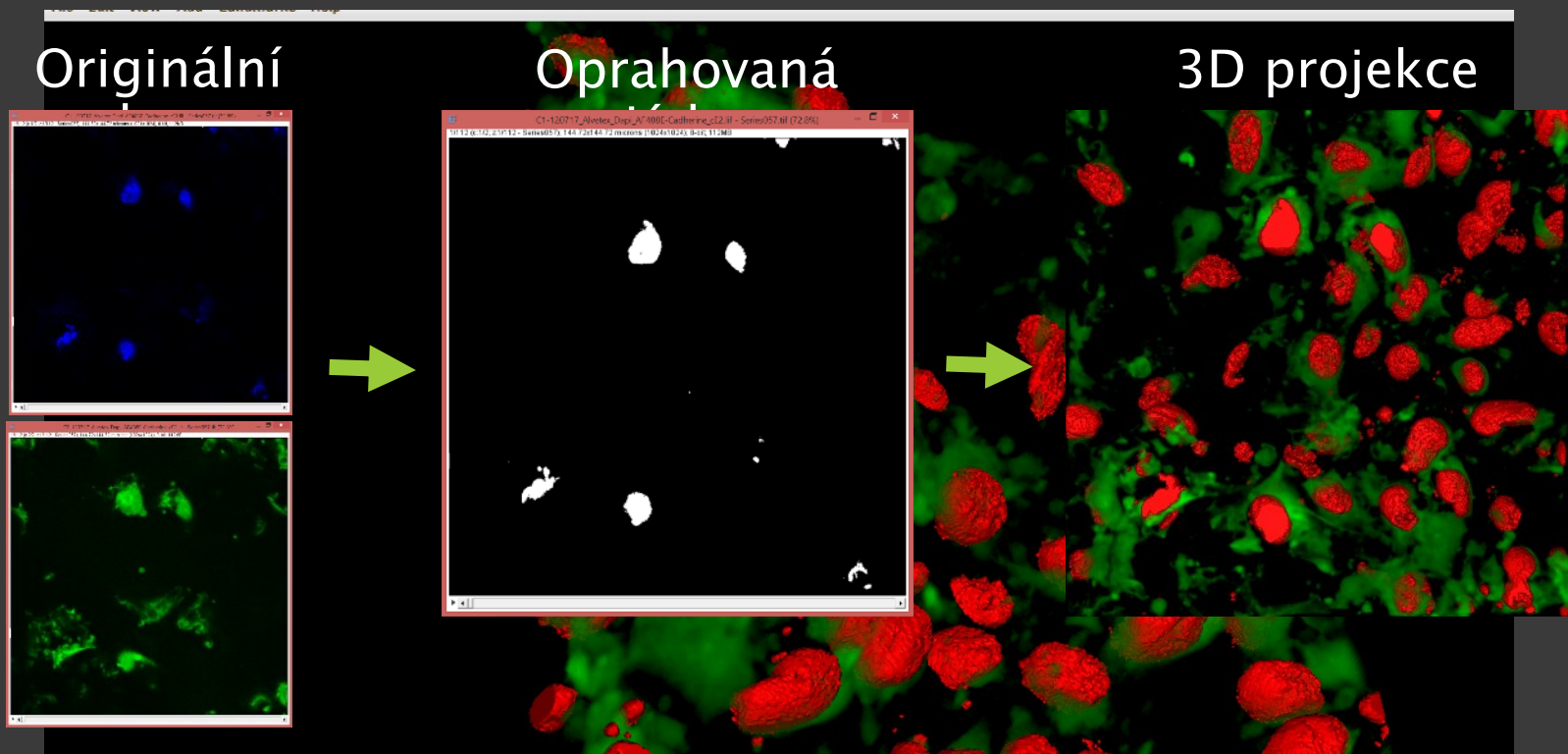
Watershed



Voda stéká do údolí

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

SEGMENTACE – 3D MODELOVÁNÍ



ANALÝZA - SOFTWARE

CellProfiler

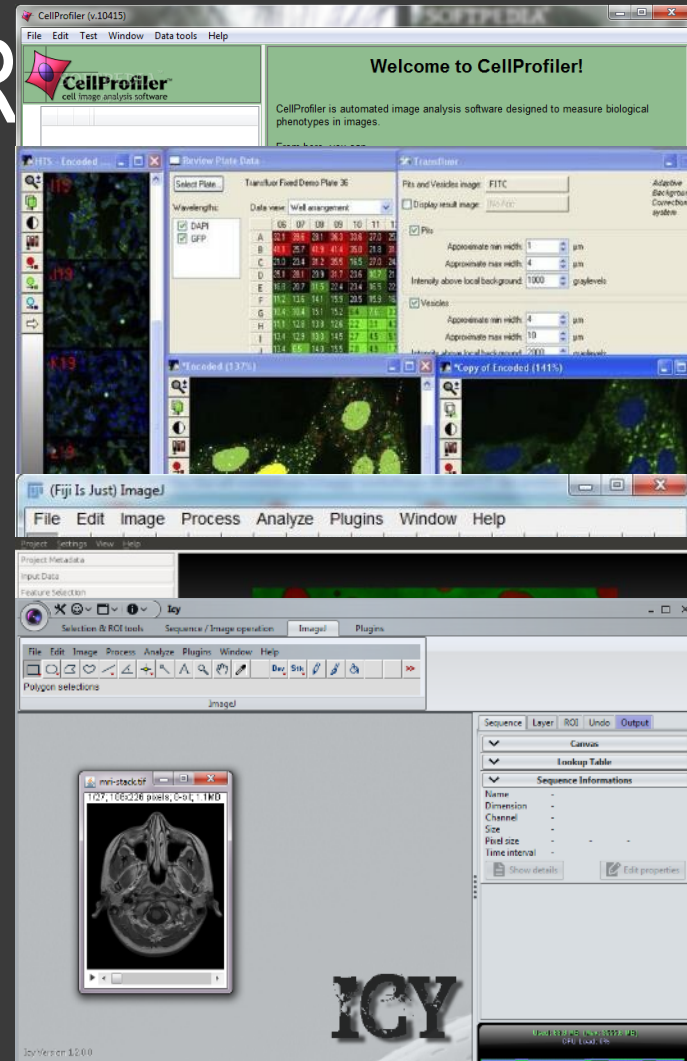
MD Metaxpress

LAS X

FIJI / ImageJ

Ilastik

Icy



ImageJ:
Image →
Properties
Analyze →
Analyze Particles

OBRAZOVÁ ANALÝZA - STEREOLOGIE

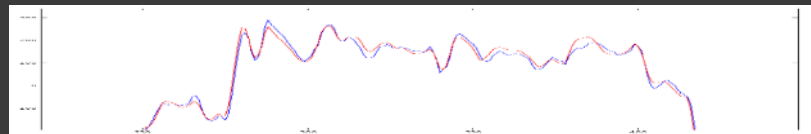
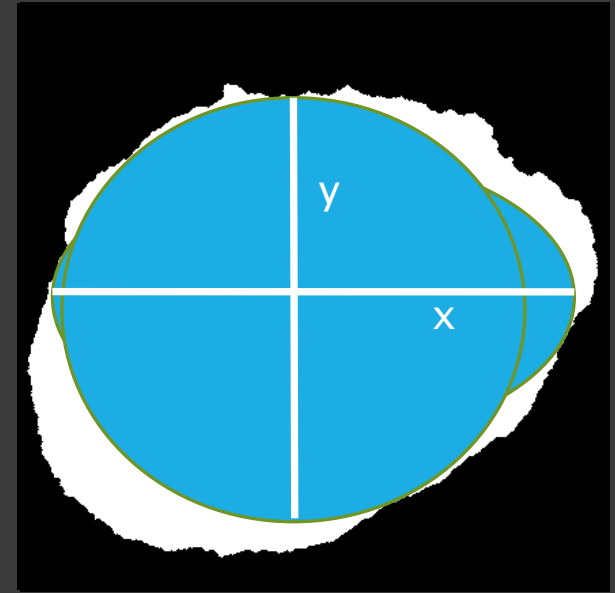
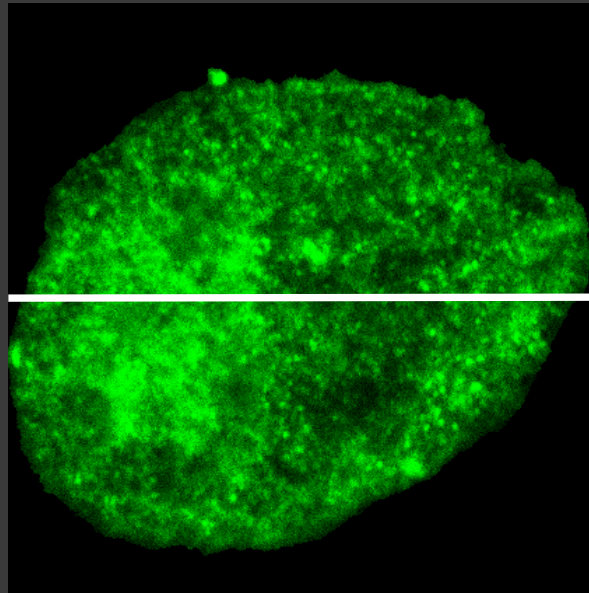
Velikost pixelu

Plocha

Intenzita (bitová
hl.)

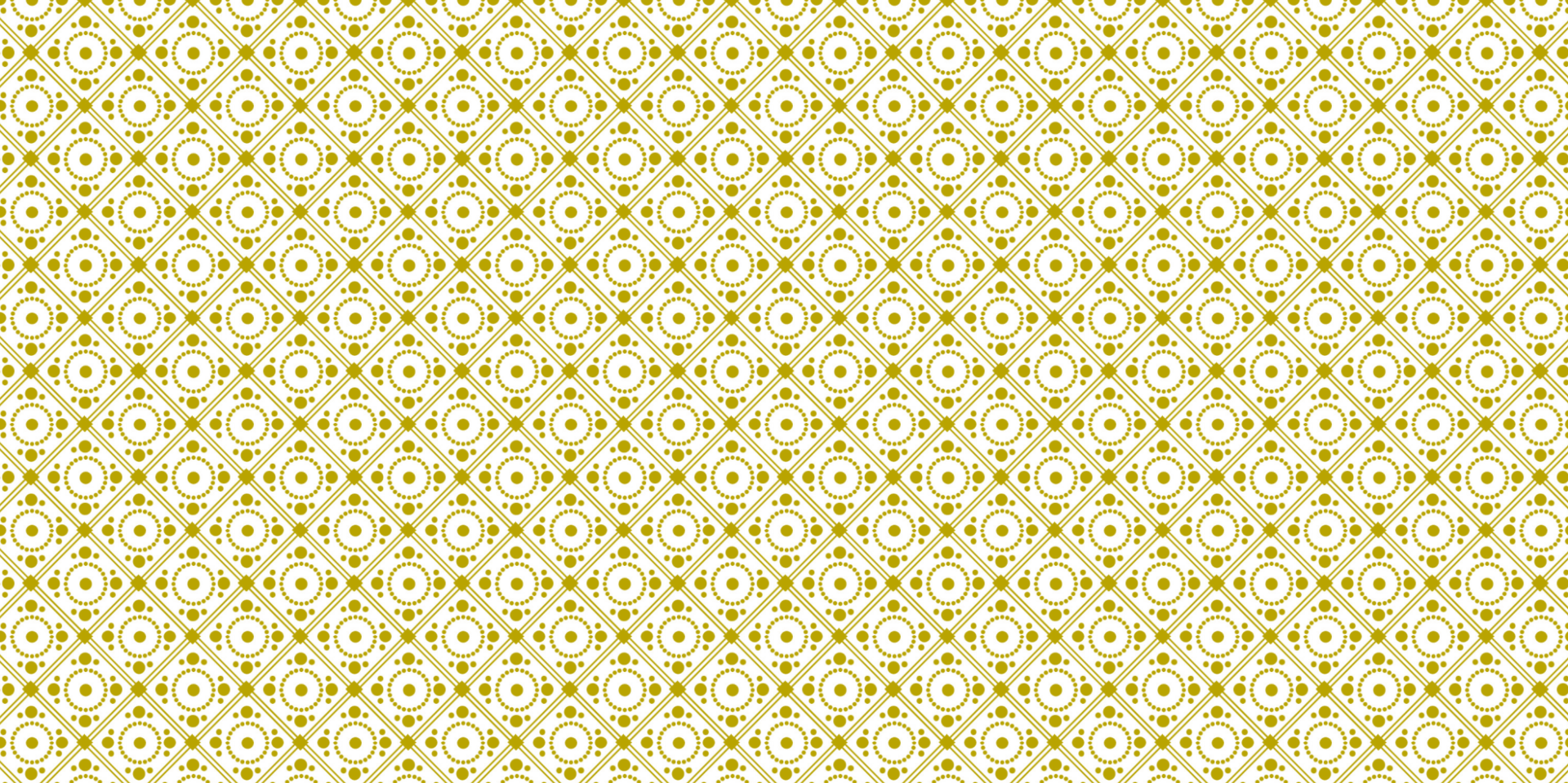
Textura

Morfologie



PROBLÉMY

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

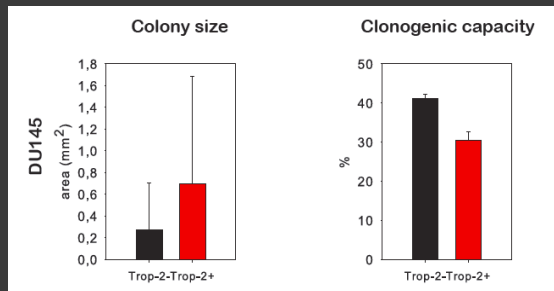
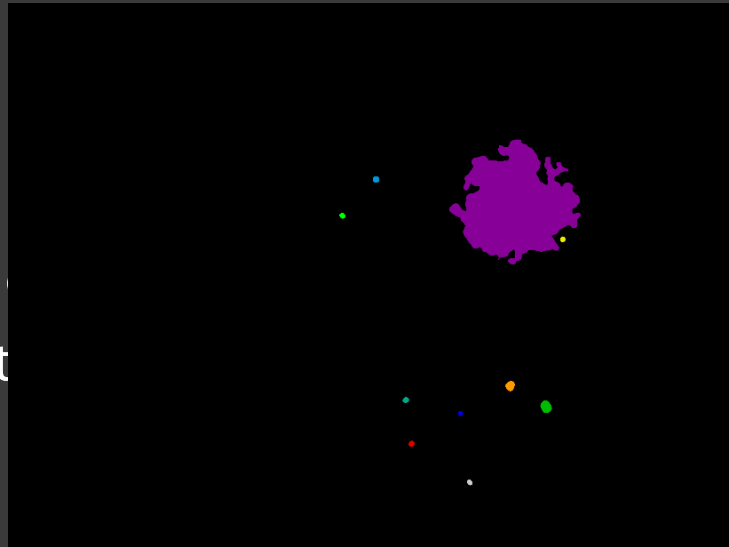


APLIKACE

Příklady analýz

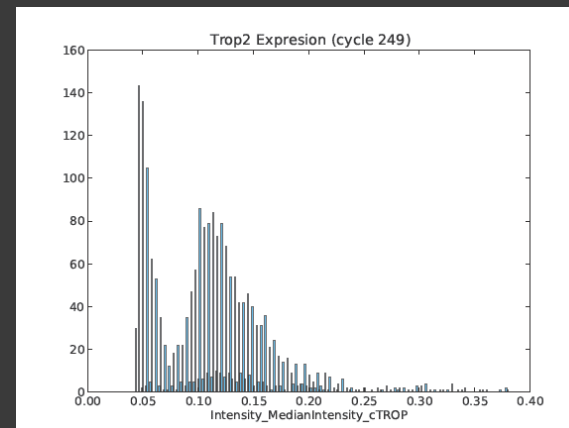
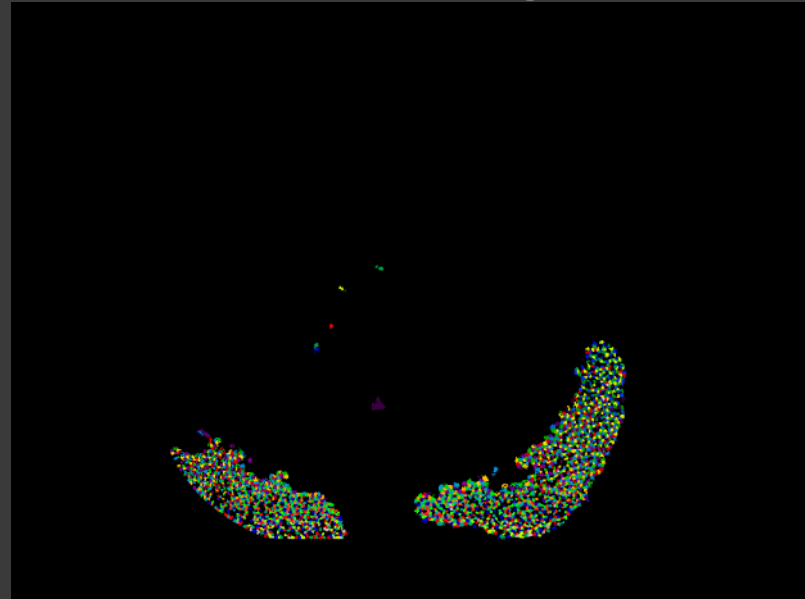
POČÍTÁNÍ BUNĚČNÝCH KOLONIÍ

- Highthroughput
- Průchozí světlo
- Velikost kolonie/plačky
- Klonogenní kapacita



KVANTIFIKACE POVRCHOVÝCH MARKERŮ

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

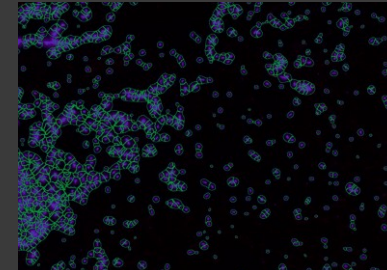
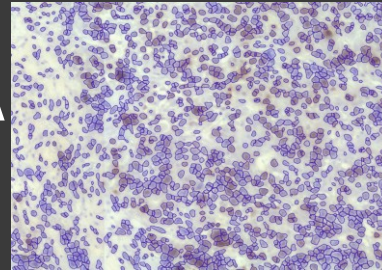


DETEKCE SFEROIDŮ A INVAZIVITY

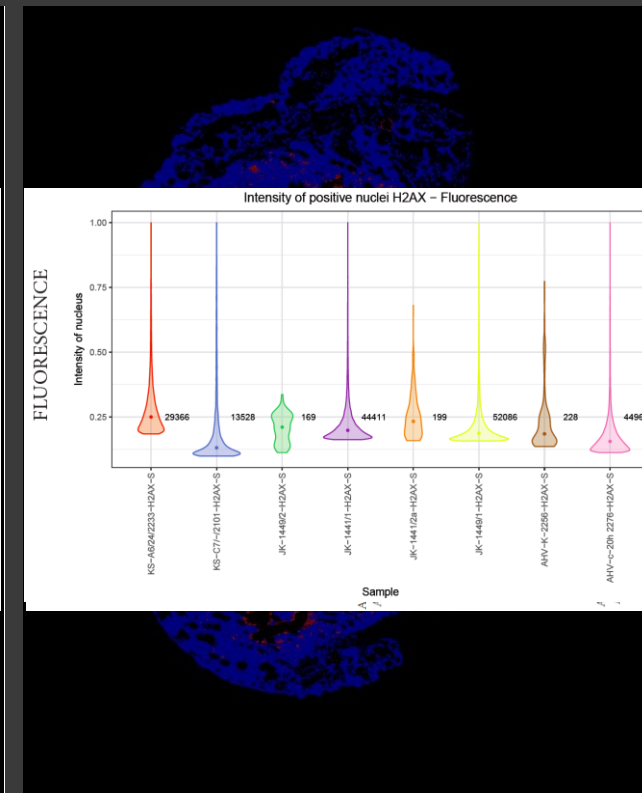
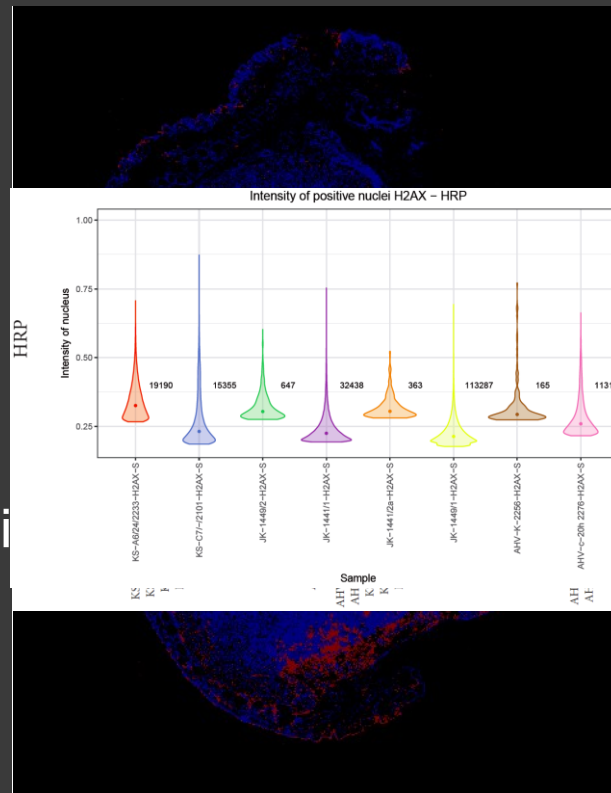
- Hightroughput
- Průchozí světlo
- Detekce sferoidů
- Detekce invadujících buněk
- Plocha



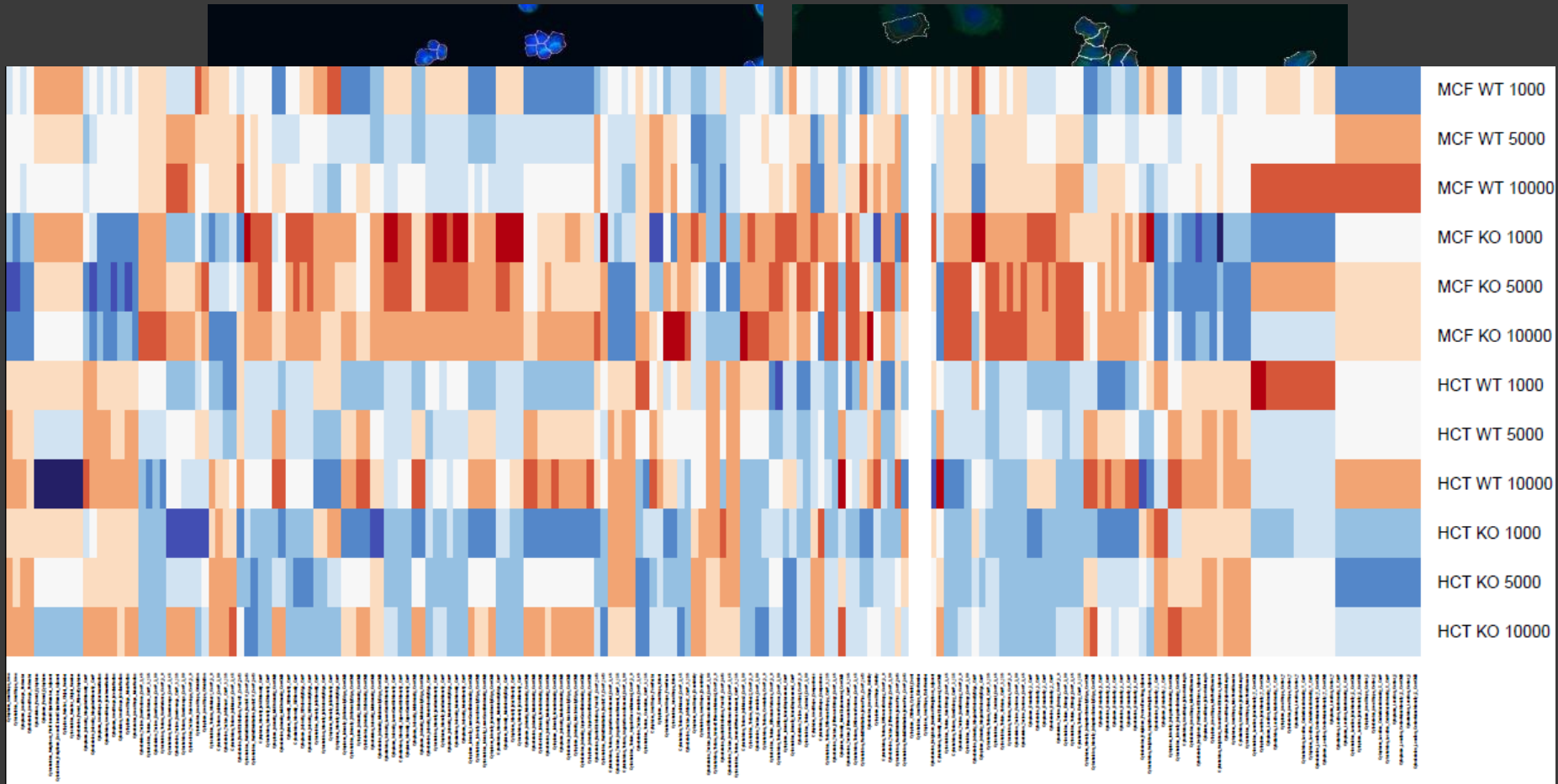
ANALÝZA TKÁ



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



ANALÝZA A KVANTIFIKACE MORFOLOGIE



IMAGEJ

FIJI

CELLPROFILER

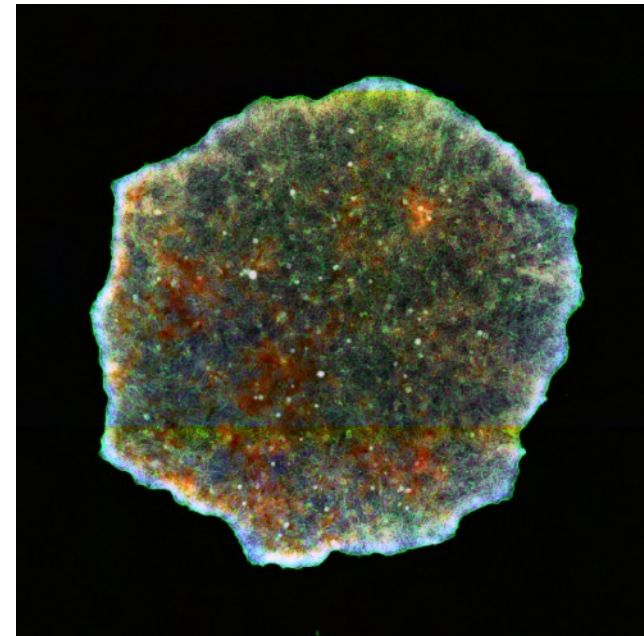
ILASTIK

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

<http://fiji.sc/>

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

<http://ilastik.org/>



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POZORNOST

28.11.2017

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