Hochschule Aalen
Hochschule Reutlingen
Reutlingen University
Syddansk Universitet
Ruprecht-Karls-Universität Heidelberg
hochschule mannheim

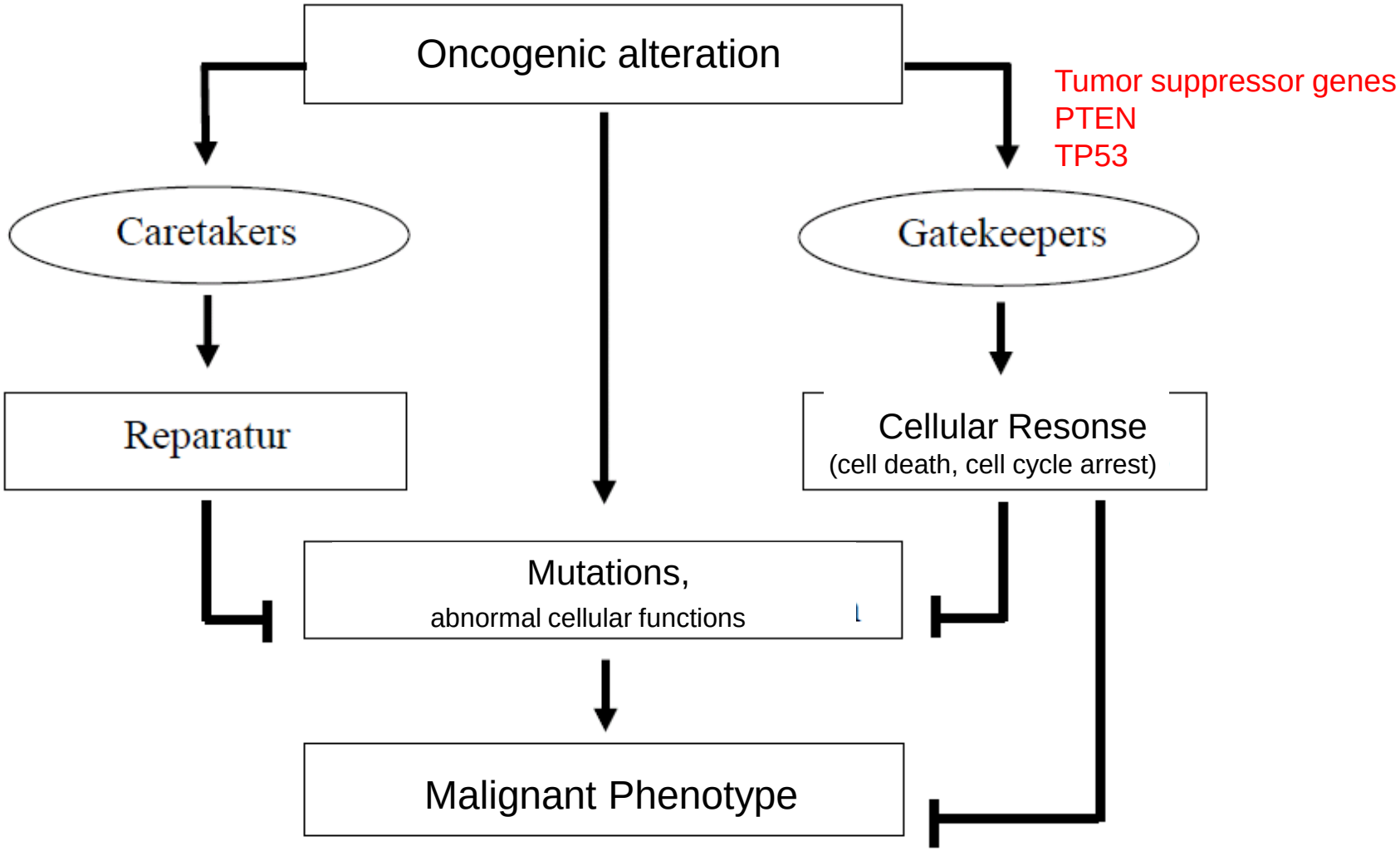
ZAFH – PHOTON ^{n}
PHOTONische Verfahren in neuen Dimensionen

Schwerpunkt 1: Multidimensionale Mikroskopie

Isogene U251-MG Glioblastom-Zellmodelle für Autofluoreszenz-Analysen

H.M. Kuhn, A. Holloschi, C. Müller, K. Bieser, S. Blaich, M.Hudler, S. Schmidt, R. Kessler, H. Schneckenburger, A. von Deimling, J. Mollenhauer, P. Kioschis

Tumor development
Glioblastoma

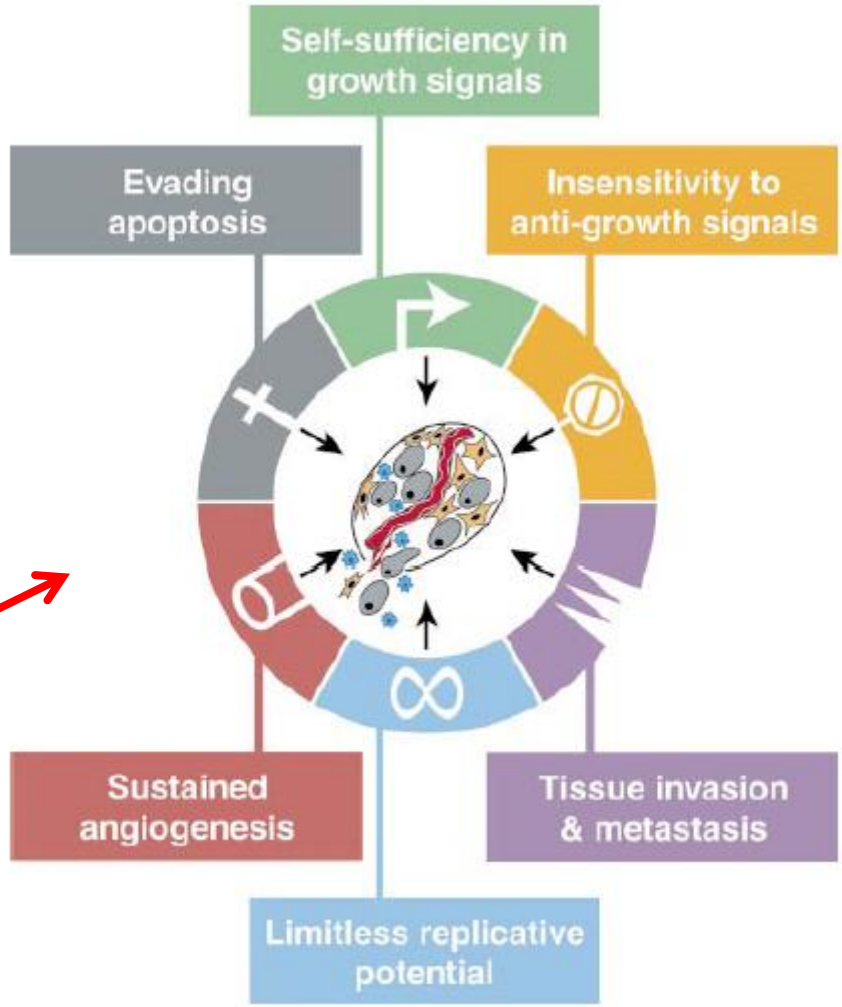


Acquired Capabilities of Cancer

- most if not all cancers have acquired the same set of of functional capabilities during their development, albeit through various mechanistic strategies.

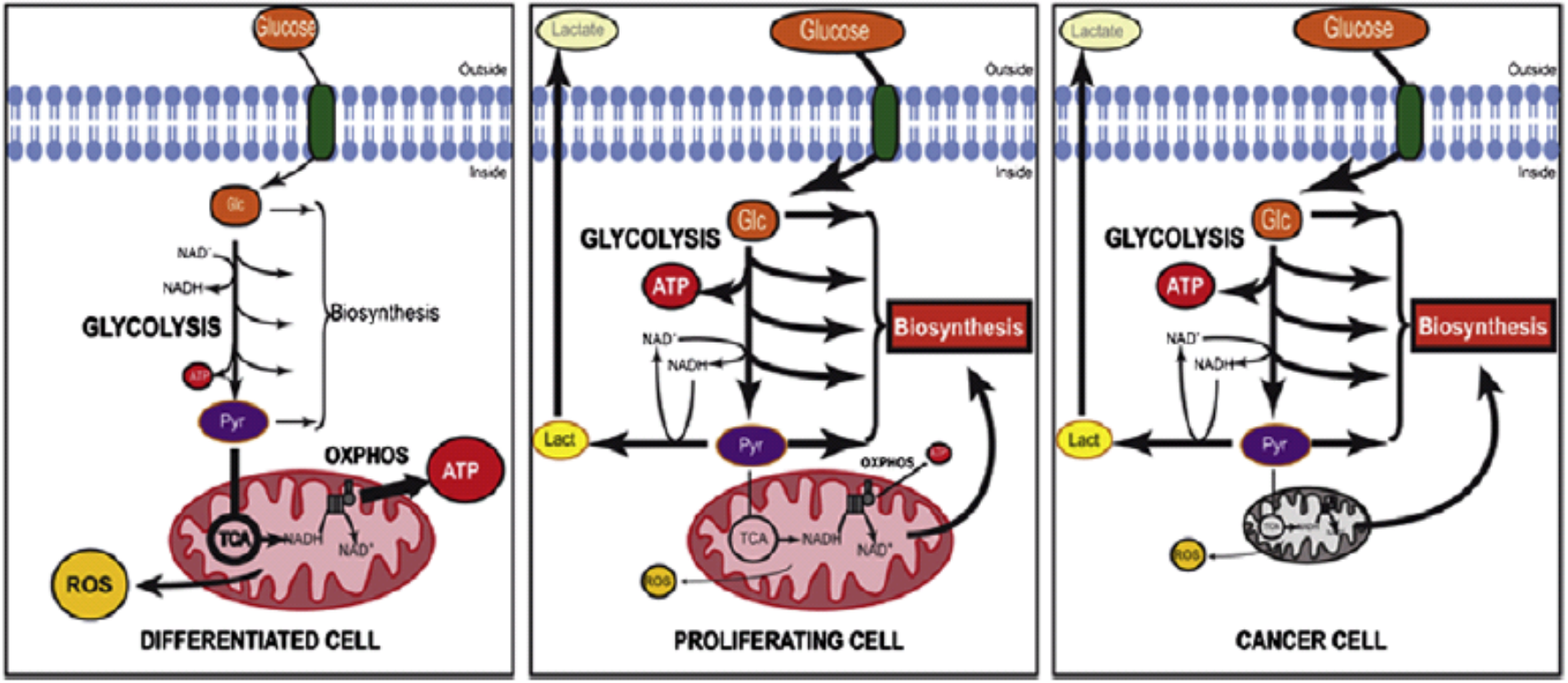
- six functional chacteristics of cancer:
  1) persistant growth signals
  2) evasion of apoptosis
  3) insensitivity to anti-growth signals
  4) unlimited replicative potential
  5) angiogenesis
  6) invasion and metastasis

+ the seventh hallmark of cancer:
  7) metabolic changes
  (characteristic metabolic signature of cancer cells)



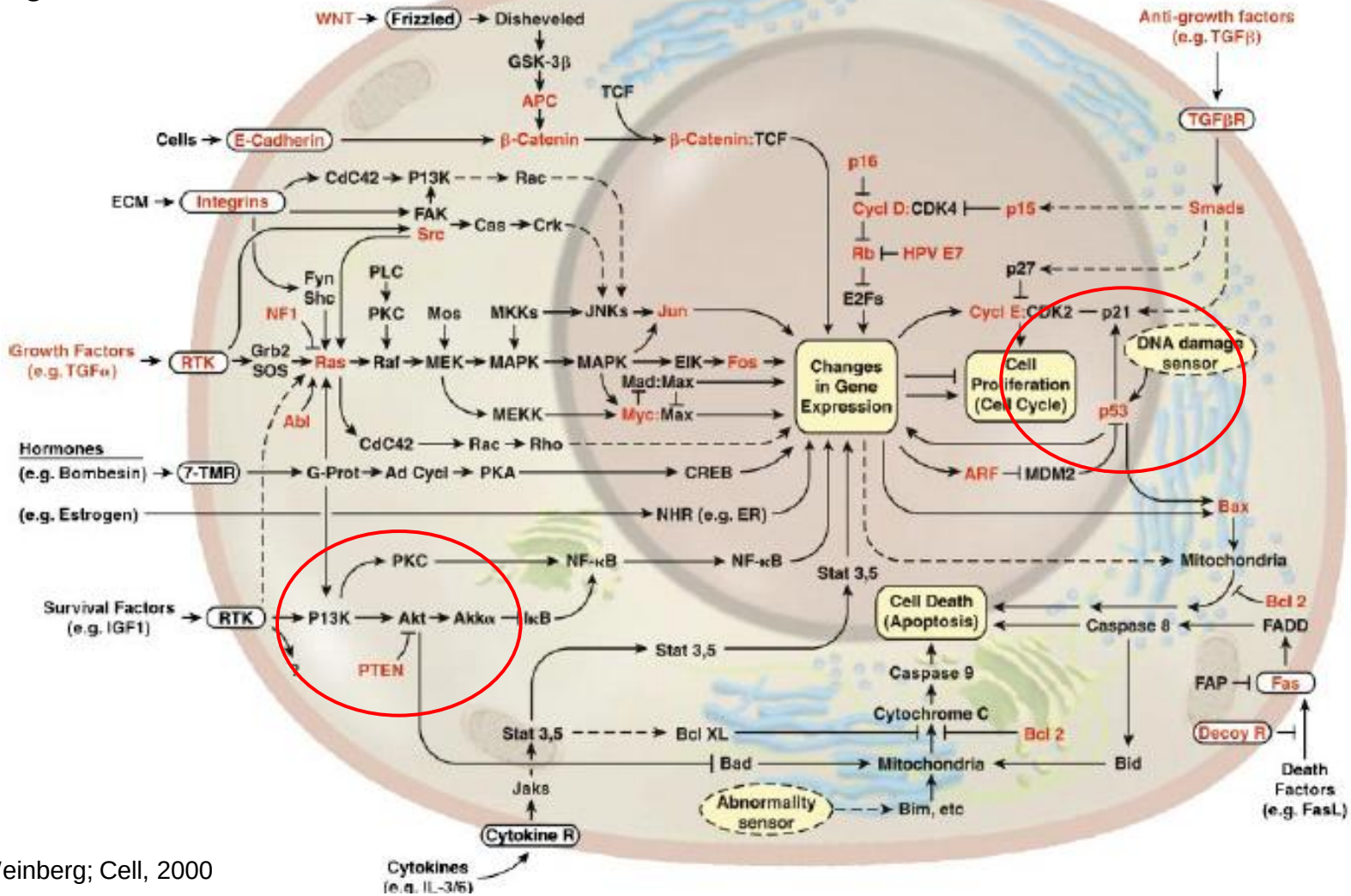
D. Hanahan, R. A. Weinberg; Cell, 2000;
Yeung et al., Cell. Mol. Life Sci 2008

Fluxes of matter and energy in differentiated, proliferating and cancer cells



Proliferating and cancer cells: high demand of glucose to provide metabolic precursors for the biosynthesis of the macromolecules of daughter cells and because most of the energy required for anabolic purposes derives from non-efficient non-respiratory modes (glycolysis, pentose phosphate pathway) of energy generation

Complex signalling induces cancer

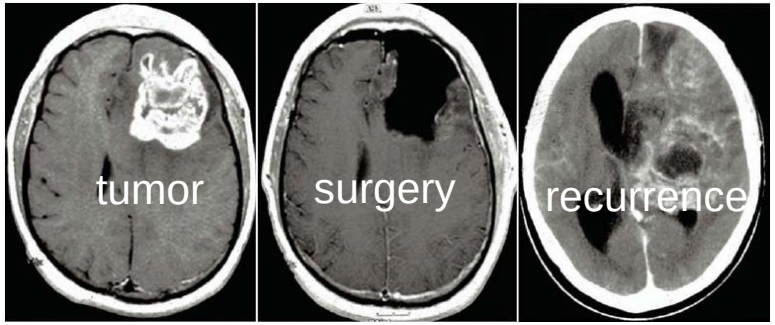


D. Hanahan, R. A. Weinberg; Cell, 2000

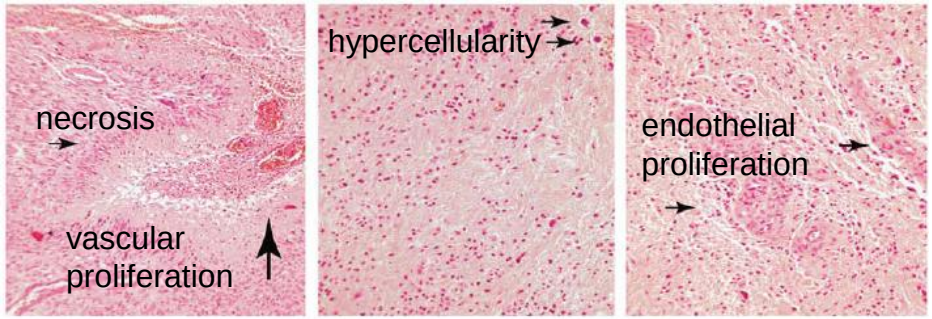
Glioblastoma Multiforme (GBM)

- most common primary tumors of the brain
- incidence of about 25,000 new cases per year in the United States
- patients with GBM have a poor prognosis, with a median survival of one year with aggressive therapy
- mainstays of treatment include surgical resection, radiation, and chemotherapy
- gliomas generally recur at the surgical resection margin(s)
- GBMs are grade IV tumors: aggressive, invasive, destructive malignancies, with increased mitotic activity, pronounced angiogenesis, necrosis, and proliferation rates two to five times higher than grade III tumors
- Most de novo GBMs: no alterations in TP53; nearly all carry EGF receptor (EGFR) gene amplifications
- a lowgrade to a high-grade glioma often involves the serial accumulation of genetic alterations that inactivate tumor suppressor genes—such as TP53, p16, RB, PTEN —or activate oncogenes such as MDM2, CDK4 and CDK6

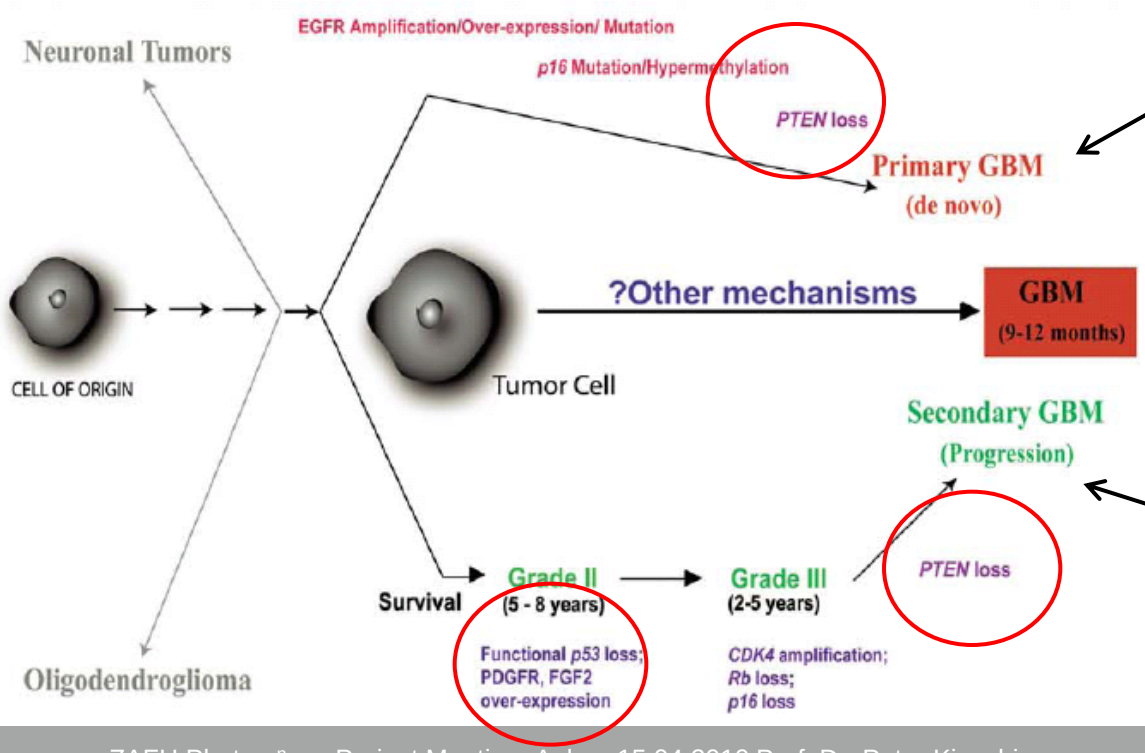
Tumor development
Glioblastoma



neuroimages from a patient with glioblastoma multiforme



Histopathological examples of patient's tumor



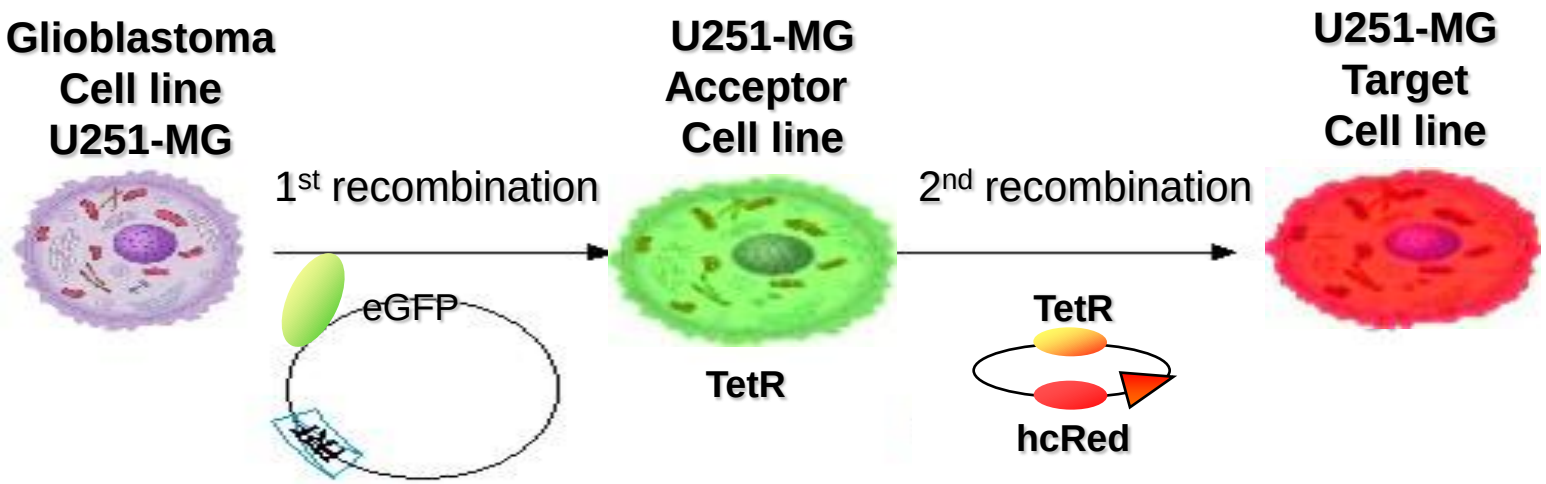
growth factor signaling
cell proliferation,
cell cycle progression,
apoptosis inhibition

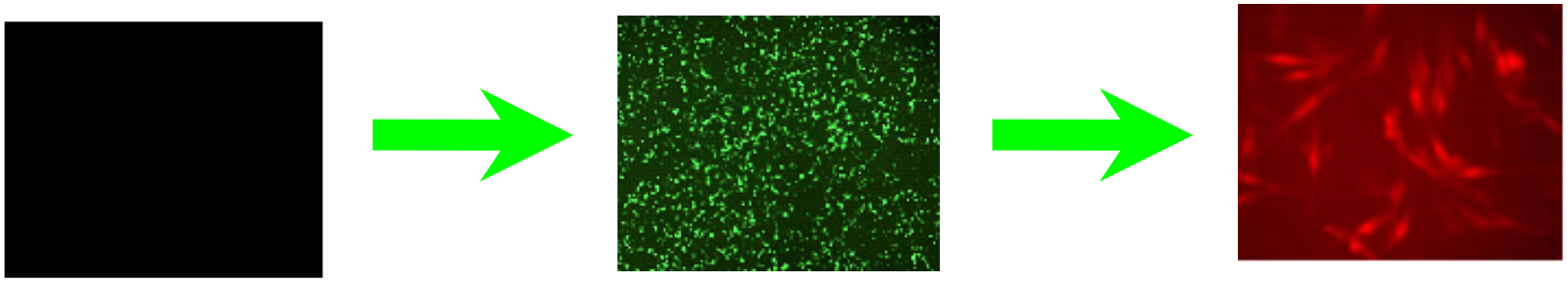
direct dys-regulation of
cell cycle arrest

Weil, PloS Medicine, 2006

Recombination efficiency

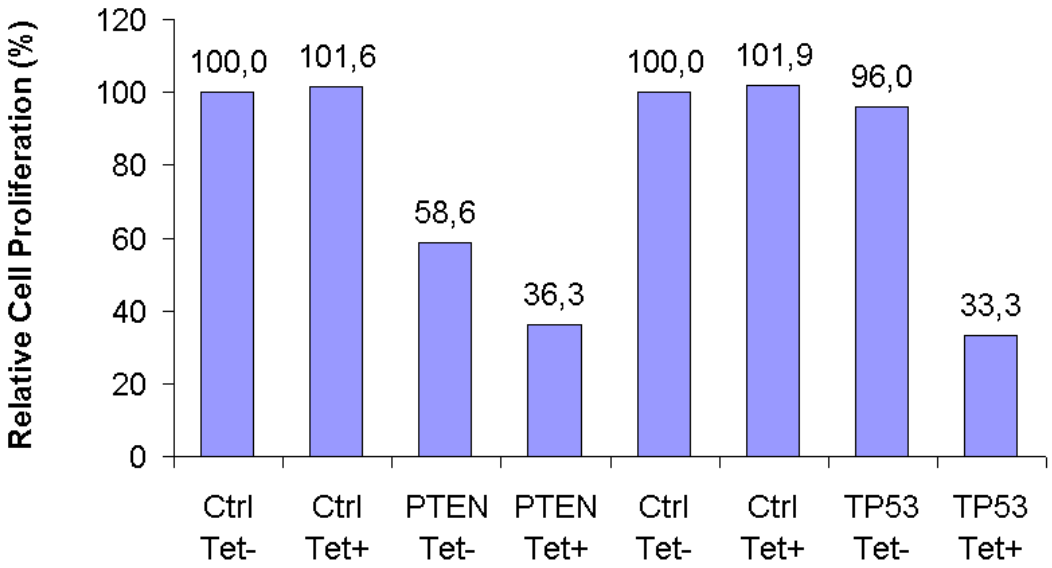
How many cells express the recombinant gene / protein?





Isogenic U251-MG

leaky promoters proliferation rates

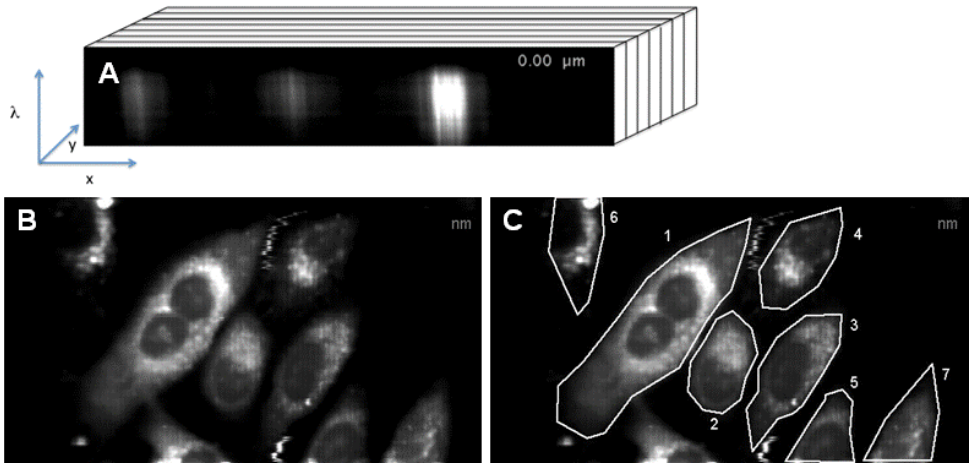


- Ctrl: Control; recombinant U251-MG with empty plasmid; deficient for PTEN and TP53
- PTEN/TP53: isogenic U251-MG; recombinant for either PTEN or TP53 (Tet-On inducible)
- “leaky” tet promoters: leaky but low expression of PTEN results in significant reduction of proliferation
- TP53: in comparison to PTEN more TP53 is necessary for proliferation (tumor) suppression
- Can qualitative and quantitative differences be measured?

Isogenic U251-MG
Do analysed cells express PTEN / TP53?

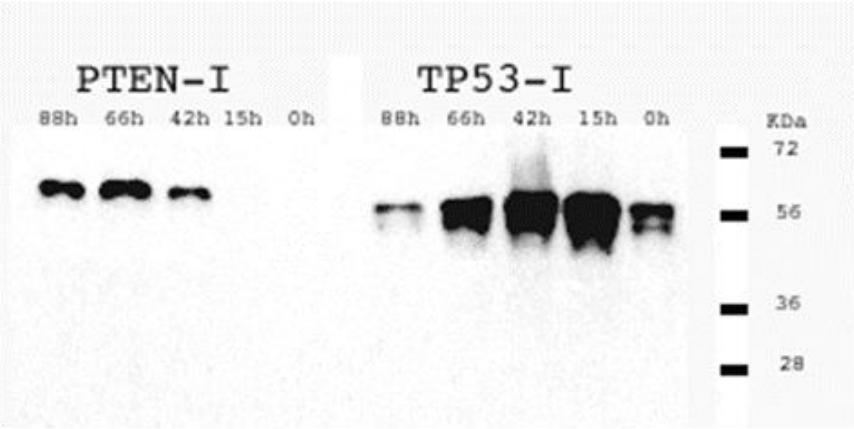
Single cell analysis
Protein expression

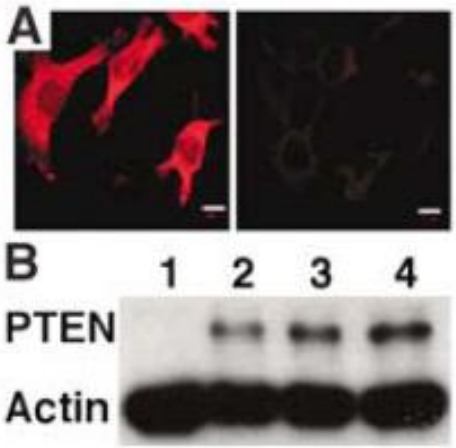
Spectral Imaging
(excitation 750nm, 800 nm, 850nm and 880 nm)



measurements of approx. 7-10 cells

Western-blot analysis (parallel)
verification of protein expression

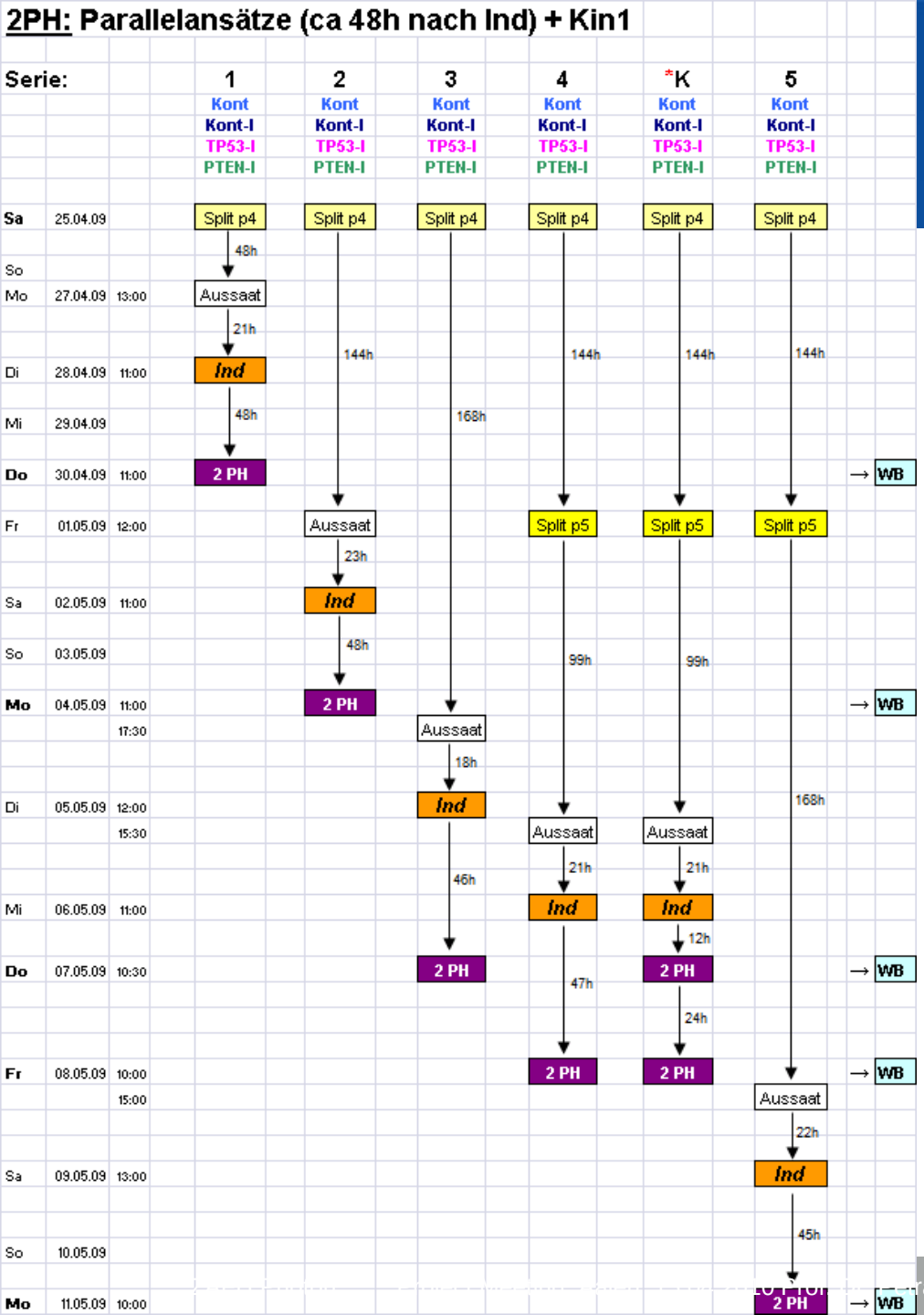




Aim → Parallel immunocytochemical detection in cells and at western blot

Isogenic U251-MG

Spectral analysis 48 h induction

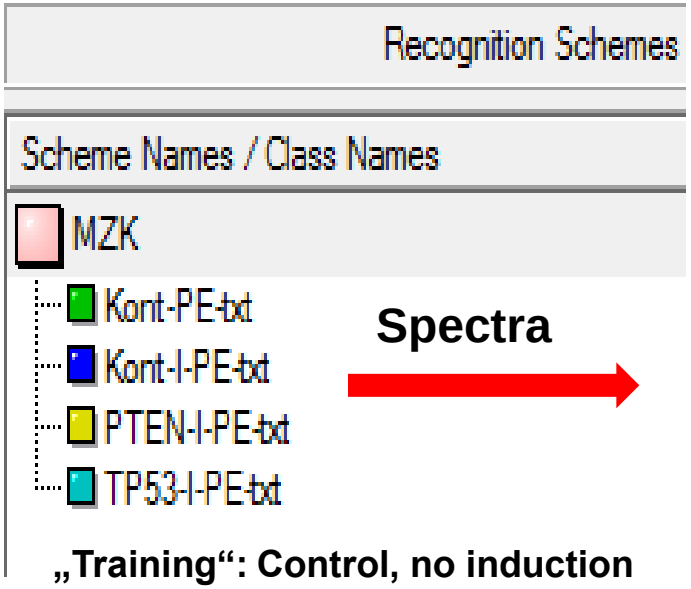


- Measurements after 48h Tet –induction
- 7 – 10 cells / measurements
- 750nm, 800 nm, 850nm and 880 nm
- Mean values for each wavelength / single cell

Kont-PE-txt	21 spectra
Kont-I-PE-txt	27 spectra
PTEN-I-PE-txt	28 spectra
TP53-I-PE-txt	30 spectra

Data used for **training** of PATTERN EXPERT program

PATTERN EXPERT and software airspect
(**A**daptive **I**nformation Extraction and **R**esearch System for **Spect**roscopy)



Spectra

„Training“: Control, no induction
Control, induction
PTEN, induction
TP53, induction

Control
AcceptorCL + empty plasmid

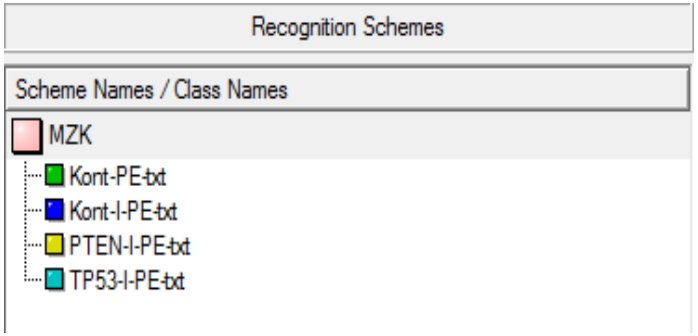
Kont-PE-txt non-induced
Kont-I-PE-txt induced

PTEN/P53
AcceptorCL
+ PTEN or P53

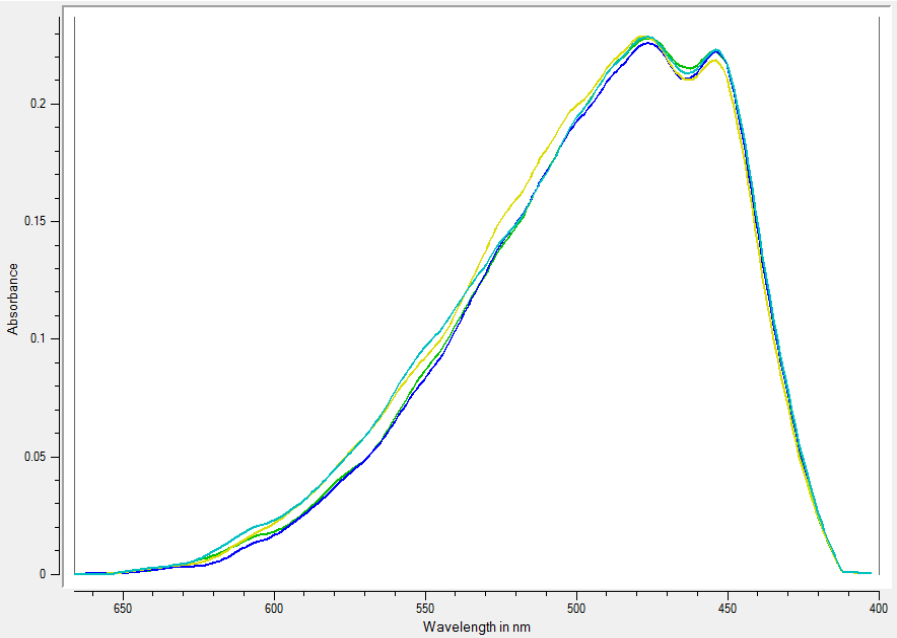
PTEN-I-PE-txt Non-induced
TP53-I-PE-txt induced

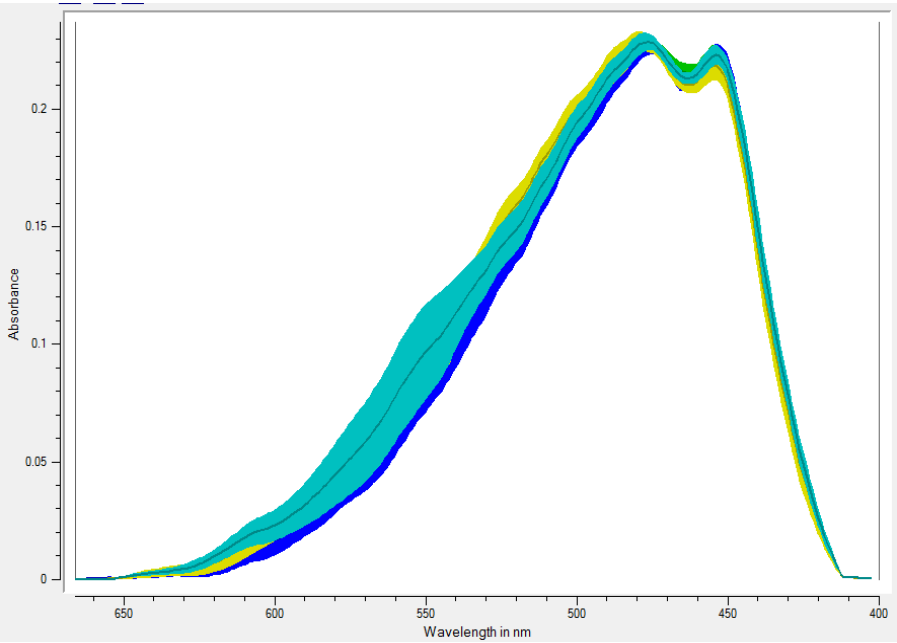
Isogenic U251-MG

Analysis of spectra

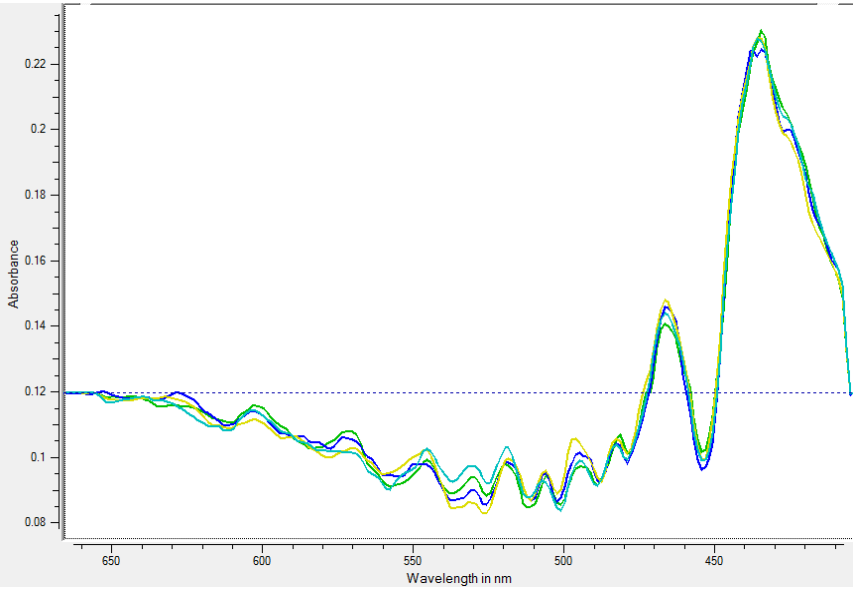


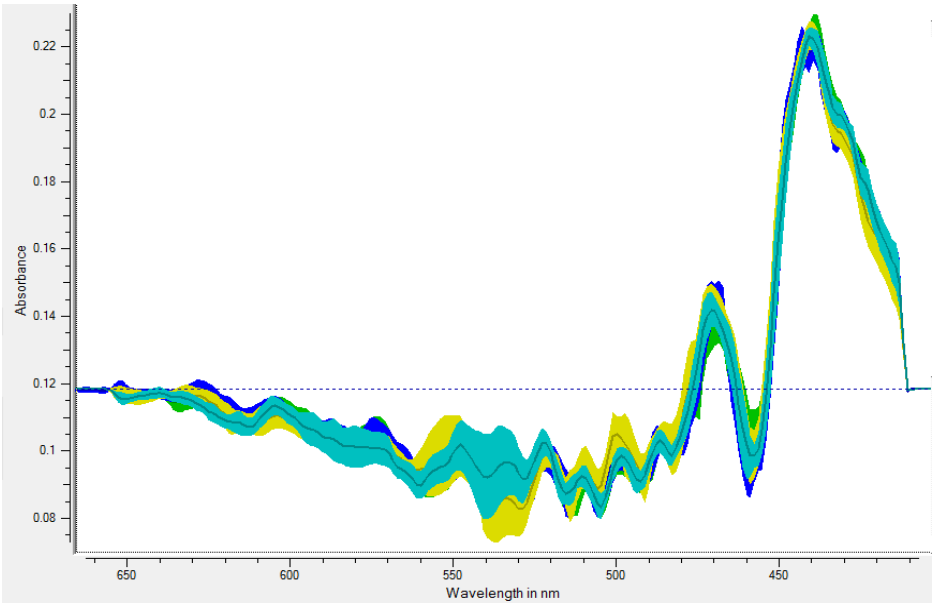
Mittelwert-Spektren



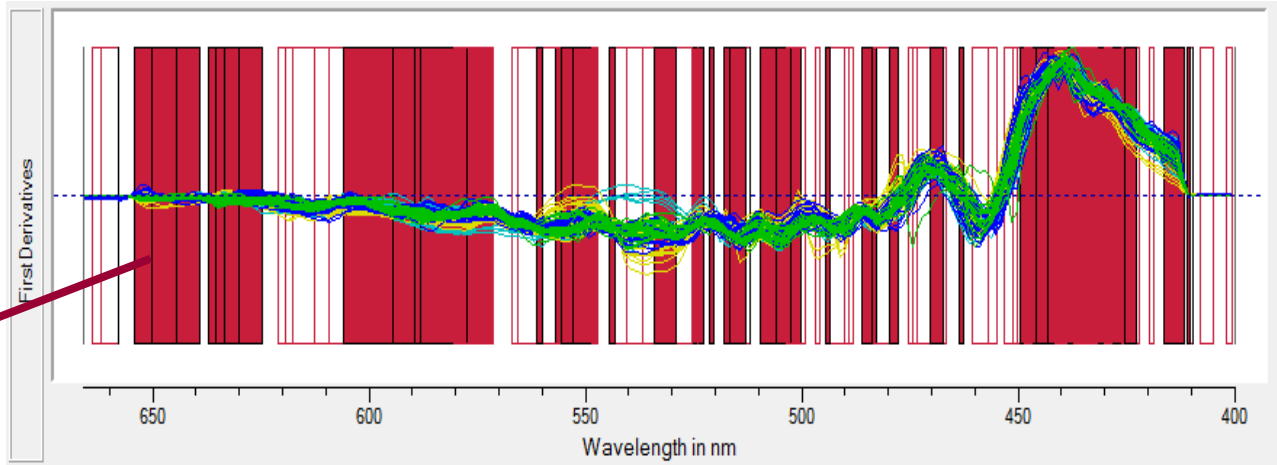


1. Ableitung der Mittelwert-Spektren

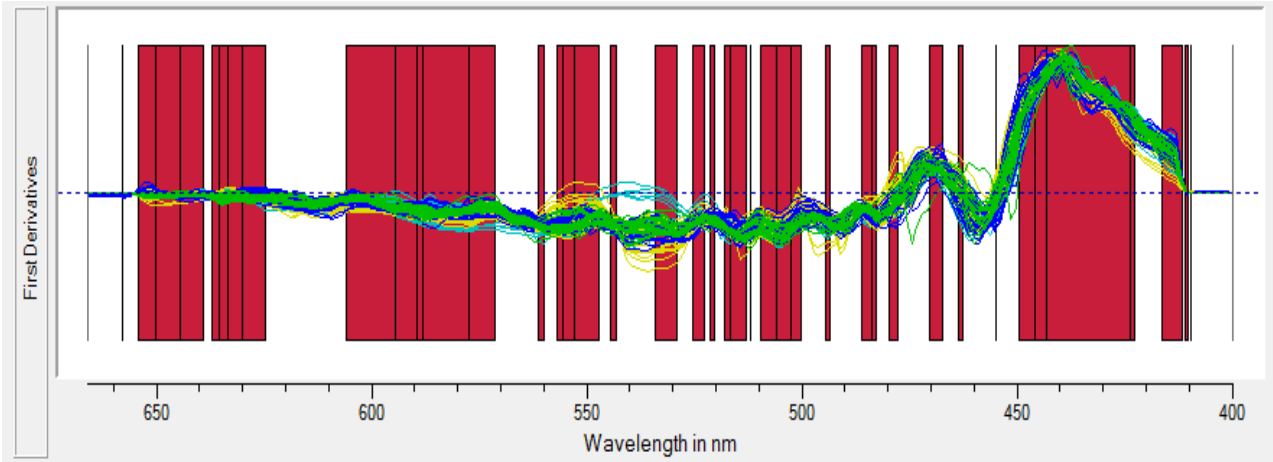




Regions of interest (ROI) defined by EP-program



- Spectral ROI pattern defined by training
- Used for classification of cell specific spectra



Self-testing neuronal network

- Internal control of each spectra and classification

Classification Result					
	Kont-PE-txt	Kont-I-PE-txt	PTEN-I-PE-txt	TP53-I-PE-txt	sum
Kont-PE-txt	21 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	21
Kont-I-PE-txt	1 (3.7%)	25 (92.6%)	0 (0.0%)	1 (3.7%)	27
PTEN-I-PE-txt	0 (0.0%)	2 (7.1%)	25 (89.3%)	1 (3.6%)	28
TP53-I-PE-txt	1 (3.3%)	0 (0.0%)	0 (0.0%)	29 (96.7%)	30
sum	23	27	25	31	106

- **accuracy between 90% to 100%**

Kinetik 2: Doxy-Puls

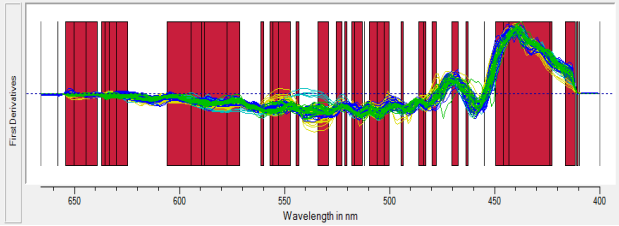
		h	h Ind	Kont	TP53	PTEN	
Sa	13:00	0		Aussaat	Aussaat	Aussaat	
	17:00	4					
	21:00	8					
So	1:00	12					
	5:00	16					
	9:00	20					
	13:00	24					
	15:00	26		DMEMØ	DMEMØ	DMEMØ	
	17:00	28					
	19:00	30					
	20:00	31		Med	Med	Med	
Mo	00:00	35					
	04:00	39					
	08:00	43					
	11:00	46		Null	Null	Null	
				Med	Med	Med	
	15:00	50					→ WB
	19:00	54					
	20:00	55	0	Ind	Ind	Ind	
Di	00:00	59					
	04:00	63					
	08:00	67					
	11:00	70	16	18h	16h	17h	→ WB
				Med	Med	Med	
	15:00	74					
	19:00	78					
	23:00	82					
Mi	03:00	86					
	07:00	90					
	11:00	94	40	41.5h	40h	41h	
				Med	Med	Med	
	15:00	98	42				→ WB
	19:00	102					
	23:00	106					
Do	03:00	110					
	07:00	114					
	11:00	118	64	66h	64h	65h	
	13:00	120	67				→ WB

Spectral analysis

Induction: 0h, 17h, 41h, 65h

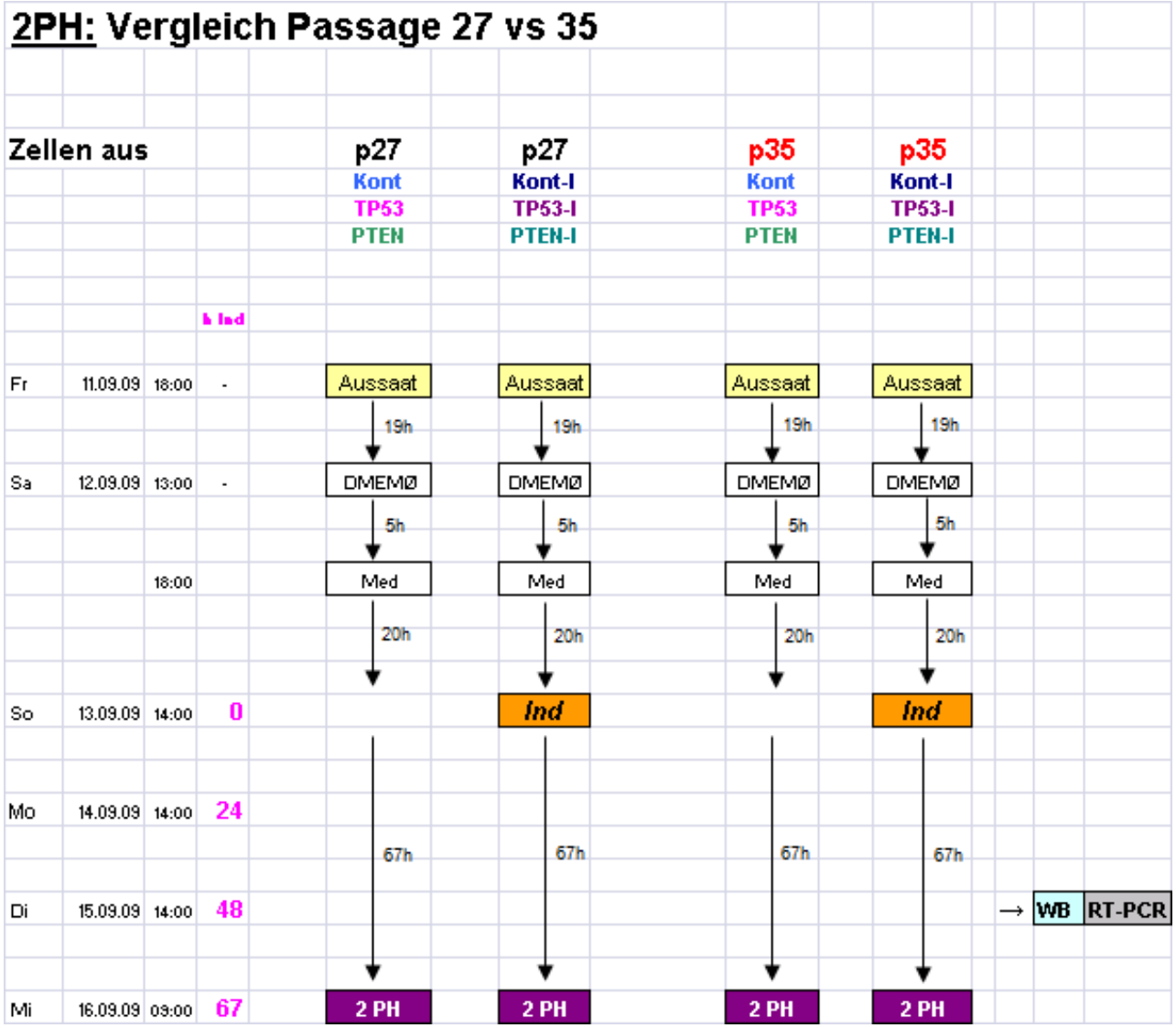
- Measurements after different Tet –induction intervalls
- 7 – 10 cells / measurements
- 750nm, 800 nm, 850nm and 880 nm
- Mean values for each wavelength / single cell

Data used in **trained** PATTERN EXPERT program



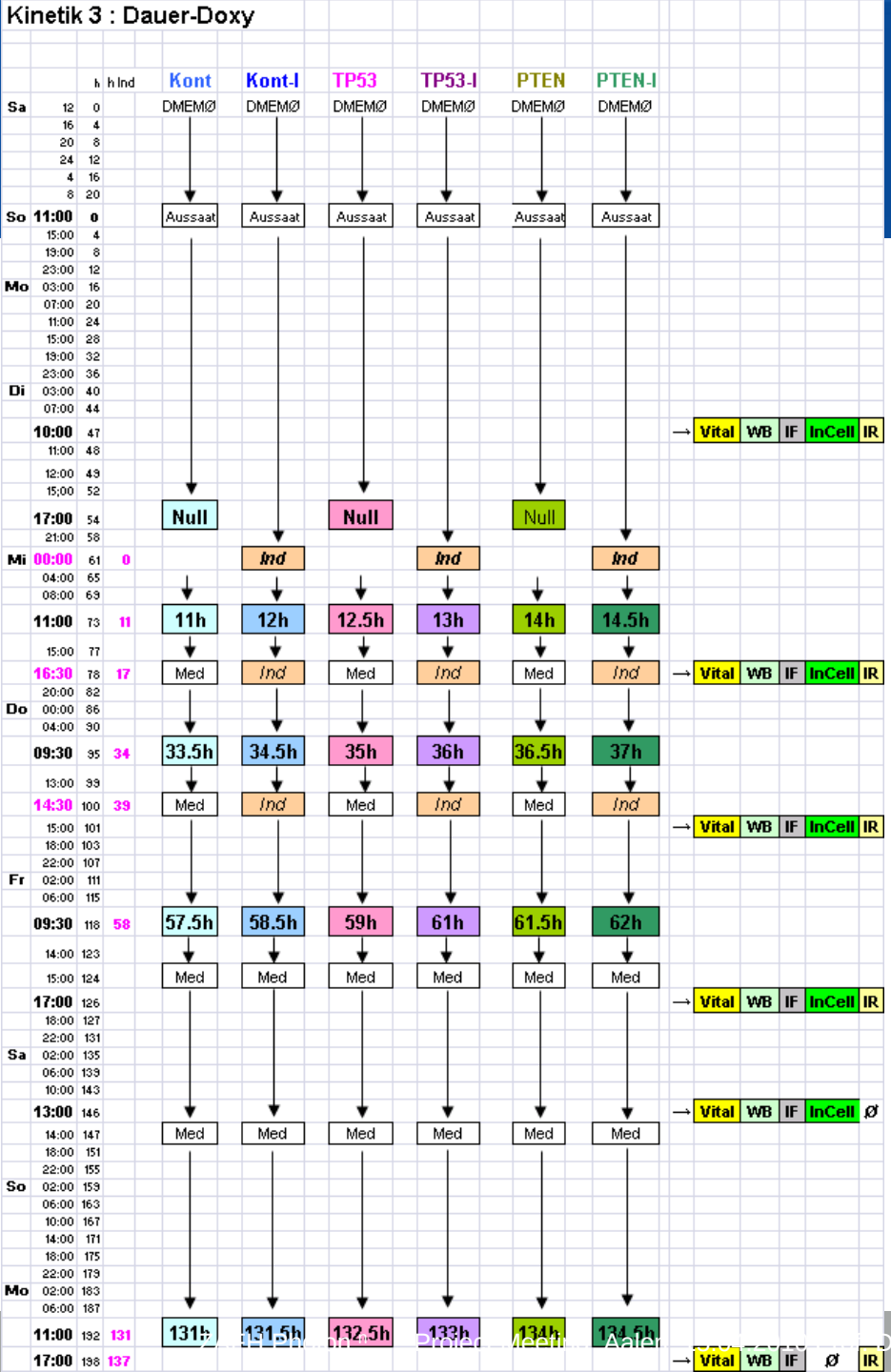
Isogenic U251-MG

Spectral analysis -passages-



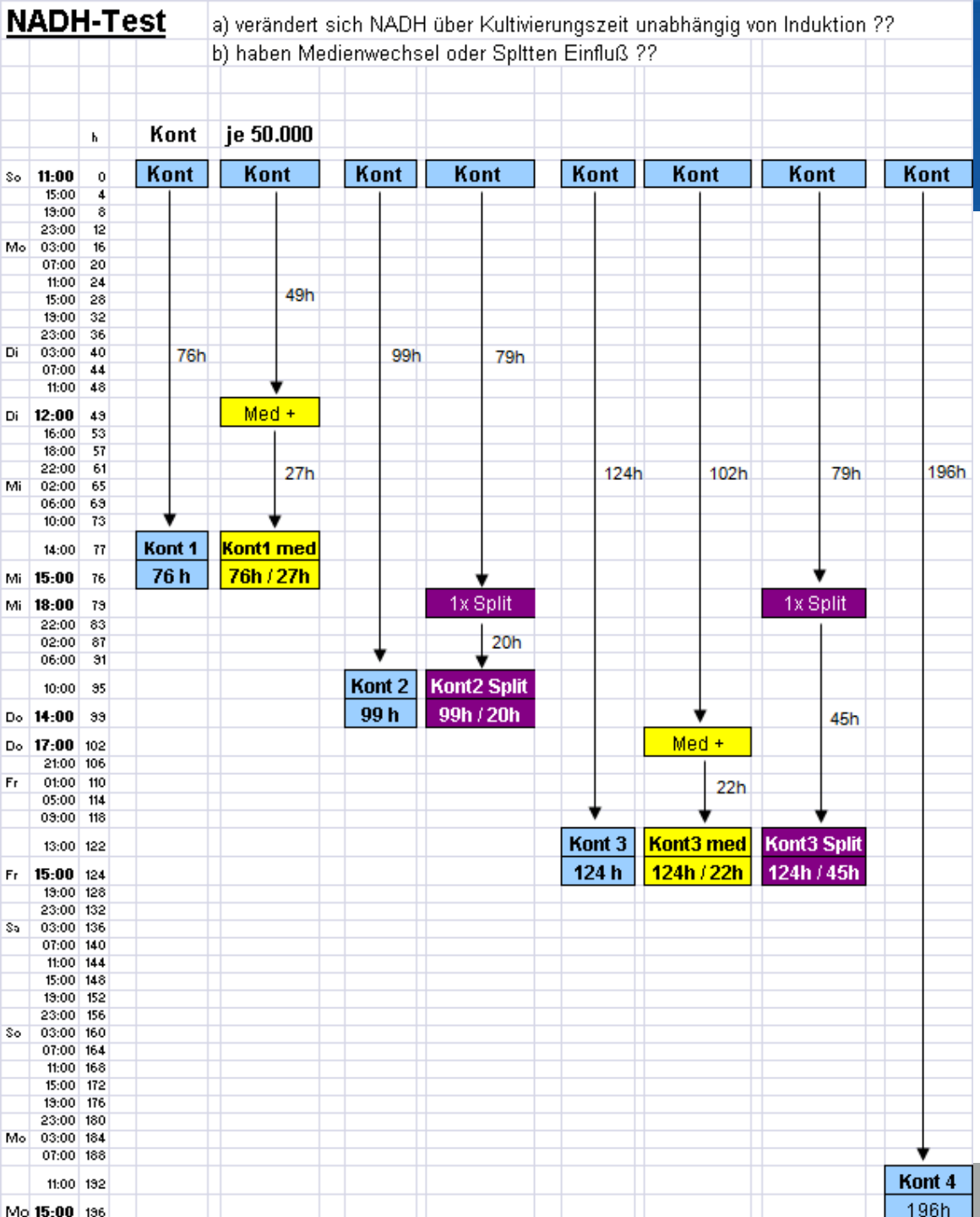
Isogenic U251-MG

Spectral analysis -constant induction-



Isogenic U251-MG

Spectral analysis -NADH-



Classification of data

Unknown Spectra to Be Recognized

	Spectrum Name	Info: File Name	MZK: Classification Result	MZK: Confidence
1	Kont-0h-1_107	Kont-0h-1.txt	PTEN-I-PE-txt	99.9
2	Kont-0h-2_108	Kont-0h-2.txt	PTEN-I-PE-txt	97.0
3	Kont-0h-3_109	Kont-0h-3.txt	PTEN-I-PE-txt	99.8
4	Kont-0h-4_110	Kont-0h-4.txt	Kont-I-PE-txt	95.9
5	Kont-0h-5_111	Kont-0h-5.txt	Kont-I-PE-txt	94.9
6	Kont-0h-6_112	Kont-0h-6.txt	PTEN-I-PE-txt	65.4
7	Kont-I-18h-1_113	Kont-I-18h-1.txt	Kont-I-PE-txt	95.6
8	Kont-I-18h-2_114	Kont-I-18h-2.txt	Kont-I-PE-txt	91.5
9	Kont-I-18h-3_115	Kont-I-18h-3.txt	Kont-I-PE-txt	97.6
10	Kont-I-18h-4_116	Kont-I-18h-4.txt	Kont-I-PE-txt	99.9
11	Kont-I-18h-5_117	Kont-I-18h-5.txt	Kont-I-PE-txt	97.2
12	Kont-I-18h-6_118	Kont-I-18h-6.txt	Kont-I-PE-txt	99.9
13	Kont-I-18h-7_119	Kont-I-18h-7.txt	Kont-I-PE-txt	89.4
14	Kont-I-18h-8_120	Kont-I-18h-8.txt	Kont-I-PE-txt	98.5
15	Kont-I-18h-9_121	Kont-I-18h-9.txt	Kont-I-PE-txt	97.6
16	Kont-I-18h-10_122	Kont-I-18h-10.txt	PTEN-I-PE-txt	53.4
17	Kont-I-41h-1_123	Kont-I-41h-1.txt	Kont-I-PE-txt	98.6
18	Kont-I-41h-2_124	Kont-I-41h-2.txt	Kont-I-PE-txt	94.8
19	Kont-I-41h-3_125	Kont-I-41h-3.txt	Kont-I-PE-txt	86.7
20	Kont-I-41h-4_126	Kont-I-41h-4.txt	Kont-I-PE-txt	95.3
21	Kont-I-41h-5_127	Kont-I-41h-5.txt	Kont-I-PE-txt	93.9
22	Kont-I-41h-7_128	Kont-I-41h-7.txt	Kont-I-PE-txt	61.1
23	Kont-I-66h-1_129	Kont-I-66h-1.txt	Kont-I-PE-txt	99.2
24	Kont-I-66h-2_130	Kont-I-66h-2.txt	Kont-I-PE-txt	97.7
25	Kont-I-66h-3_131	Kont-I-66h-3.txt	Kont-I-PE-txt	93.6
26	Kont-I-66h-4_132	Kont-I-66h-4.txt	Kont-I-PE-txt	88.5
27	Kont-I-66h-5_133	Kont-I-66h-5.txt	Kont-I-PE-txt	97.4
28	Kont-I-66h-6_134	Kont-I-66h-6.txt	Kont-I-PE-txt	77.0
29	PTEN-0h-1_135	PTEN-0h-1.txt	PTEN-I-PE-txt	98.8
30	PTEN-0h-2_136	PTEN-0h-2.txt	PTEN-I-PE-txt	93.2
31	PTEN-0h-3_137	PTEN-0h-3.txt	PTEN-I-PE-txt	98.3
32	PTEN-0h-4_138	PTEN-0h-4.txt	PTEN-I-PE-txt	99.6
33	PTEN-0h-5_139	PTEN-0h-5.txt	PTEN-I-PE-txt	100.0
34	PTEN-0h-6_140	PTEN-0h-6.txt	PTEN-I-PE-txt	100.0
35	PTEN-0h-7_141	PTEN-0h-7.txt	PTEN-I-PE-txt	99.8
36	PTEN-0h-8_142	PTEN-0h-8.txt	PTEN-I-PE-txt	100.0
37	PTEN-0h-9_143	PTEN-0h-9.txt	PTEN-I-PE-txt	99.2
38	PTEN-17h-1_144	PTEN-17h-1.txt	TP53-I-PE-txt	89.9
39	PTEN-17h-3_145	PTEN-17h-3.txt	PTEN-I-PE-txt	100.0
40	PTEN-17h-4_146	PTEN-17h-4.txt	PTEN-I-PE-txt	100.0

leakiness of Tet promoter

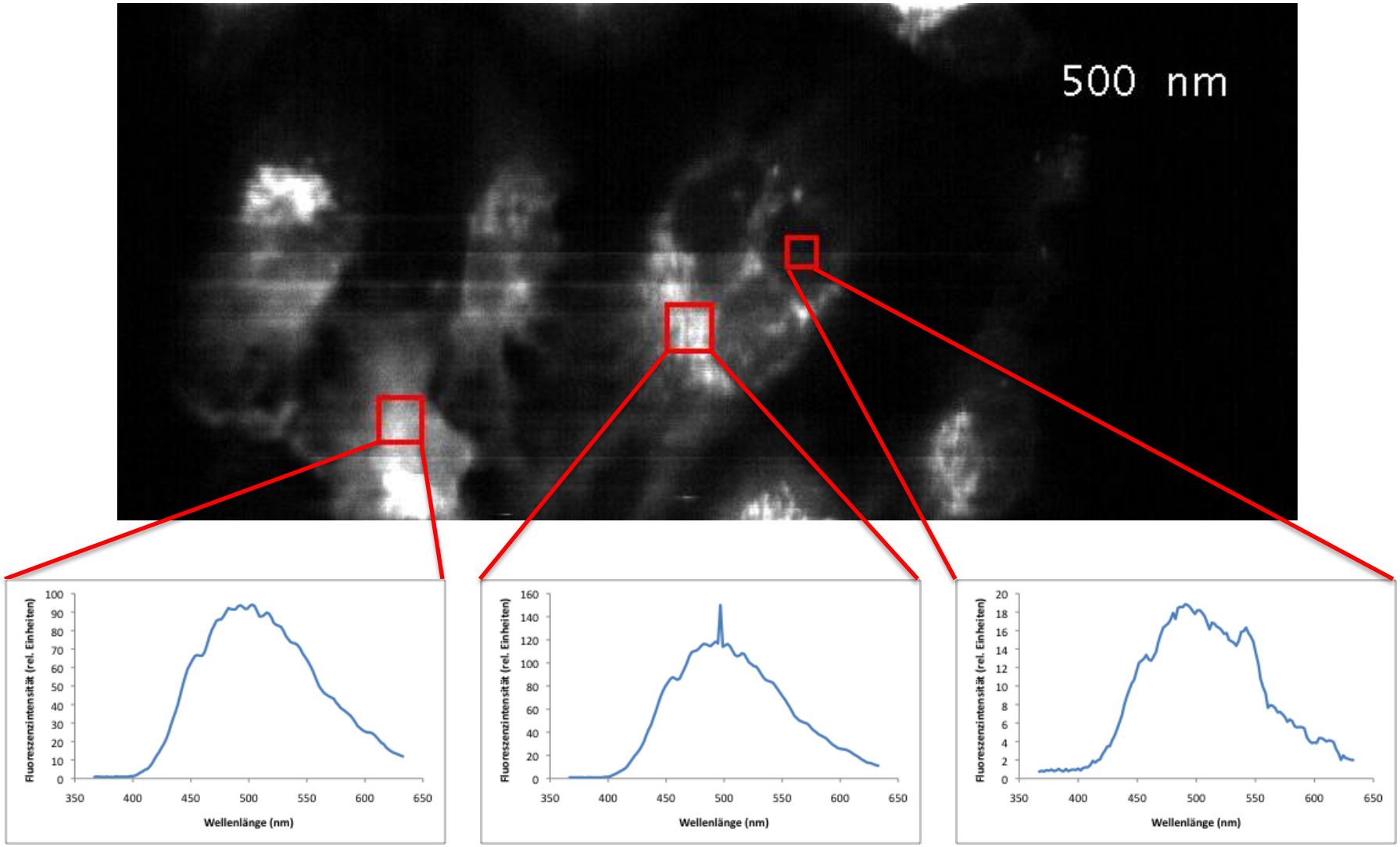
PX trained pattern

	Ctrl	TP53	PTEN
0	Ctrl		
17			
41	Ctrl-i	TP53-i	PTEN-i
65			

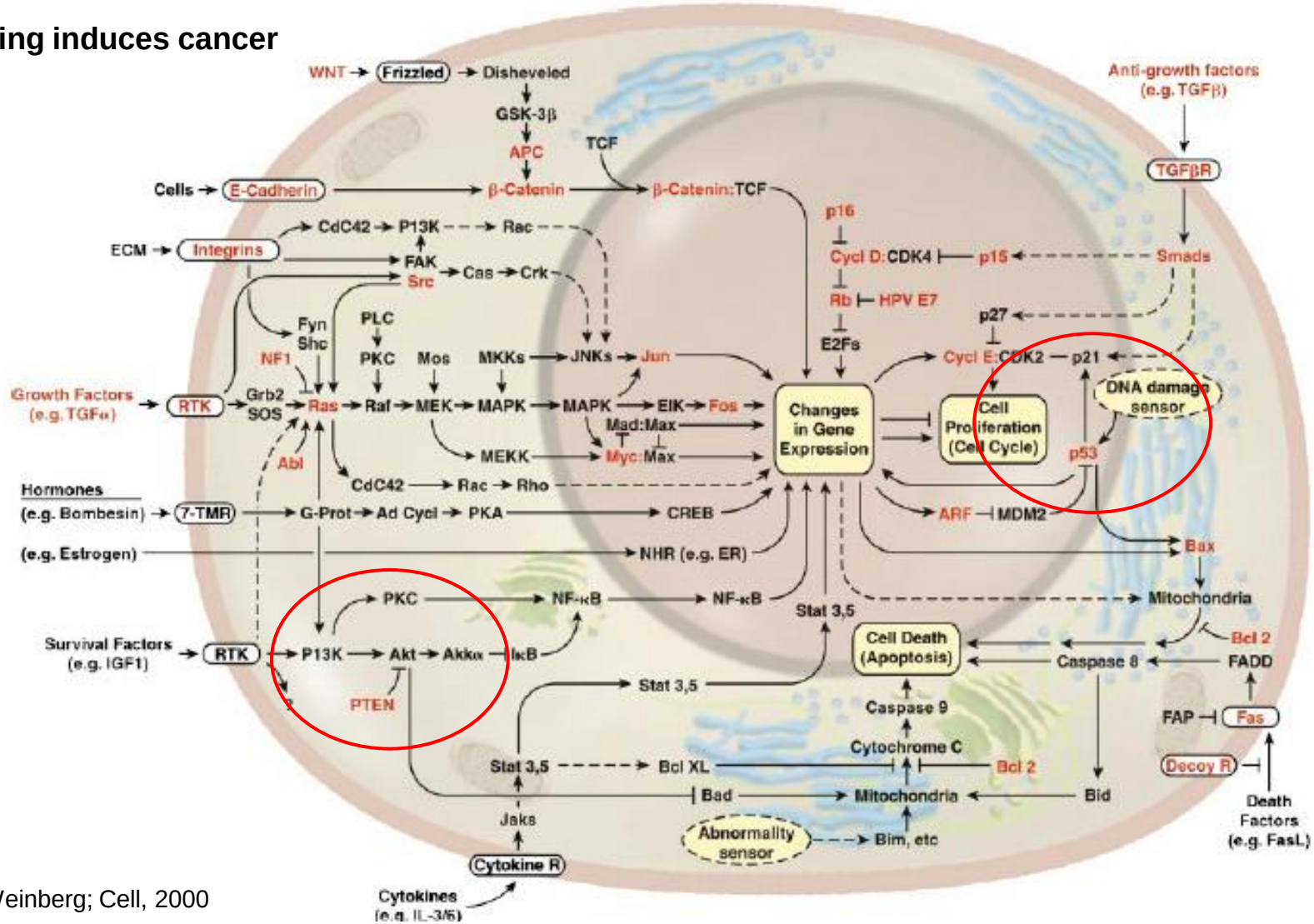
PX possible classification

	Ctrl	TP53	PTEN
0	Ctrl	Ctrl TP53-i	Ctrl PTEN-i
17	Ctrl Ctrl-i	Ctrl-i TP53-i	Ctrl-i PTEN-i
41	Ctrl-i	TP53-i	PTEN-i
65	Ctrl Ctrl-i	Ctrl-i TP53-i	Ctrl-i PTEN-i

Subcellular spectral analysis



Complex signalling induces cancer



D. Hanahan, R. A. Weinberg; Cell, 2000

Future development

Future work plan (ZAFH)

- **High resolution subcellular spectral analysis**
  (classification of differences between tumor models; analysis of higher organisation of proliferation and metabolism in cancer cells)

- **Correlation of subcellular structures with spectral analysis**
  (cell specific subcellular fluorescent markers)

- **Establishment of breast cancer isogenic cell model**

- **Spectral analysis of 3D-tumor models**
  (neurospheres, CD133+; cancer stem-like cells, cancer initiating cells)

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