hochschule mannheim

ZAFH – PHOTON n
PHOTONische Verfahren in neuen Dimensionen

Schwerpunkt 1: Multidimensionale Mikroskopie

Development of 2D and 3D isogenic cancer models: Functional analysis and spectral imaging of cellular autofluorescence

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Content

I. Spectral Imaging of Autofluorescence in 2D-Glioblastom isogenic cell models

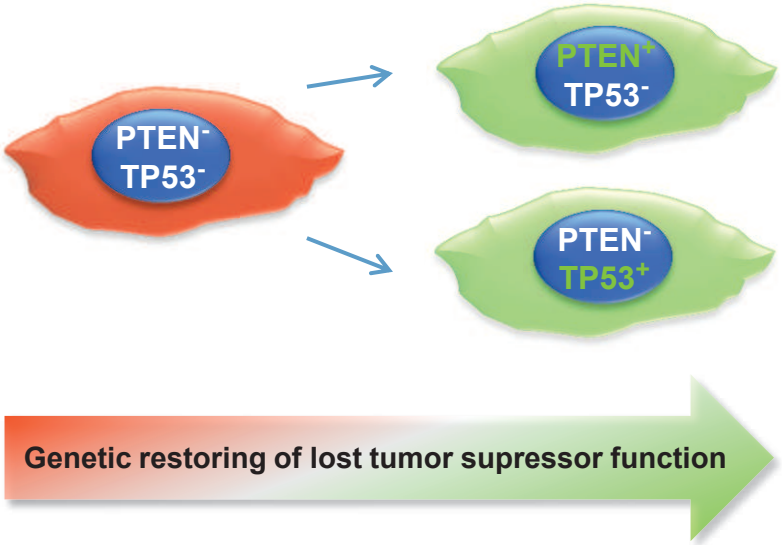
- Engineered U251-MG glioblastoma cell models
- Spectral imaging
- Data analysis and aquisition

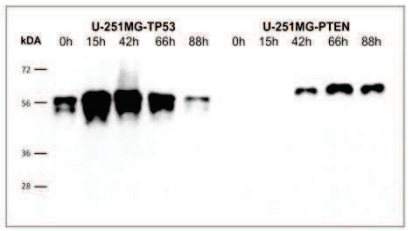
II. Establishment of standardized multicellular U251-MG spheroid models (3D)

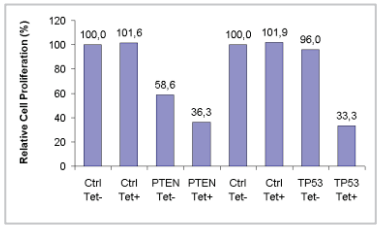
- Standardized spheroid generation
- Reproducible spheroids, determination of spheroid volume
- Spheroid functional assays
- Invasion, migration

I. Spectral Imaging of Autofluorescence in 2D-Glioblastoma Isogenic Cell Models

Application of engineered isogenic glioblastoma cells U251-MG

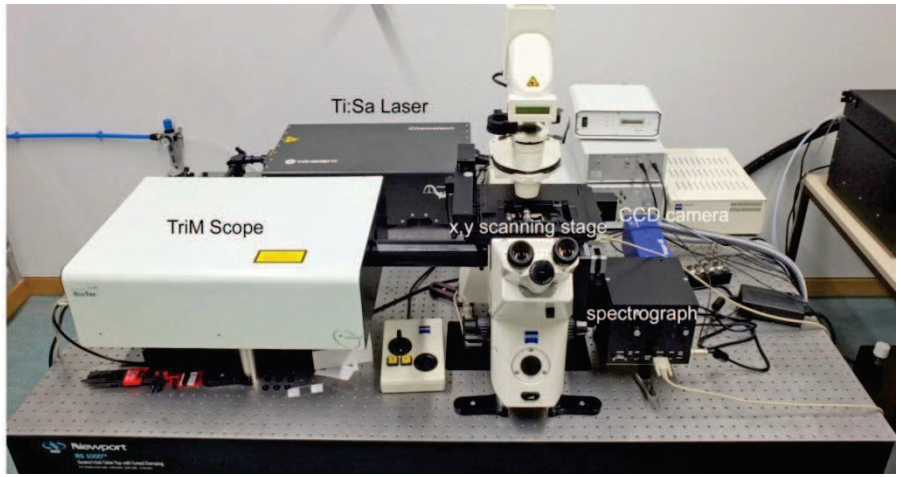


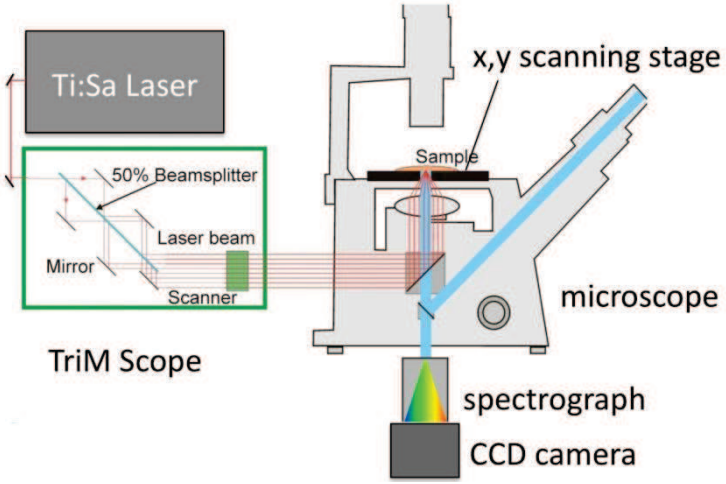




- **inducible modified tumor parameters**
  (e.g. viability, proliferation, migration, apoptosis)
- **highly standardized cell model systems for**
  - evaluation of diagnostic targets, e.g. autofluorescence
  - drug target identification
  - synthetic lethal screens for drug identification
- **standardized system for 3D cell models**
  - Multicellular Tumor Spheroids (MCTS)

Spectral Imaging of Autofluorescence



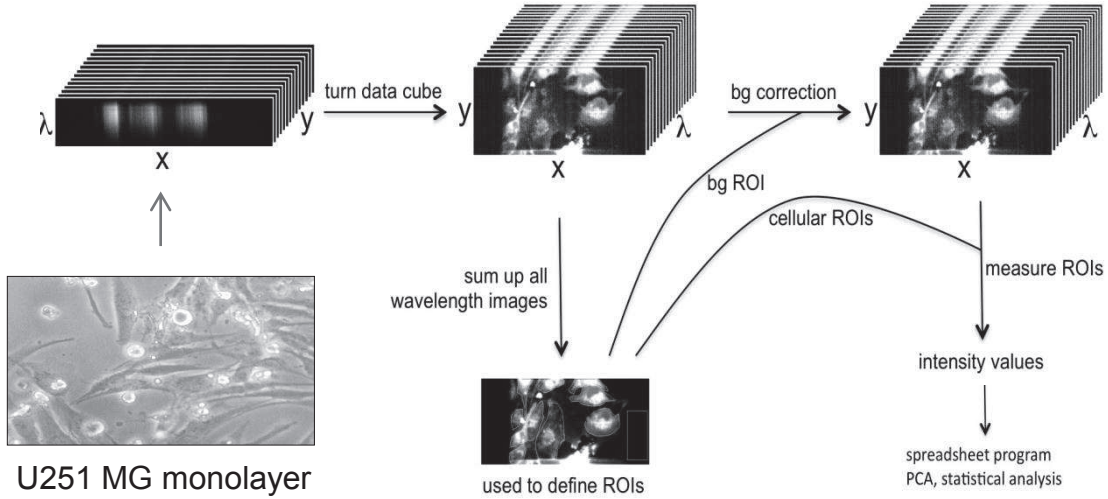


Technical set-up:

- pushbroom imaging system combined with multiphoton excitation
- tunable titanium saphire laser (Chameleon, Coherent), (120 fs pulses; 720 – 930 nm; 80 MHz)
- TriM Scope multibeam scanning device (LaVision BioTec, Bielefeld)
  - decreases acquisition time, allows higher frame rates; less cell damage
- Zeiss Axiovert 200 inverted microscope
- Acton SP2150i spectrograph (Princeton Instruments; 150 g/mm grating)
- Imager QE camera (PCO, Kelheim)
- motorised x,y scanning table (Zeiss)
- ImSpector 4.0 software (LaVision BioTec, Bielefeld).

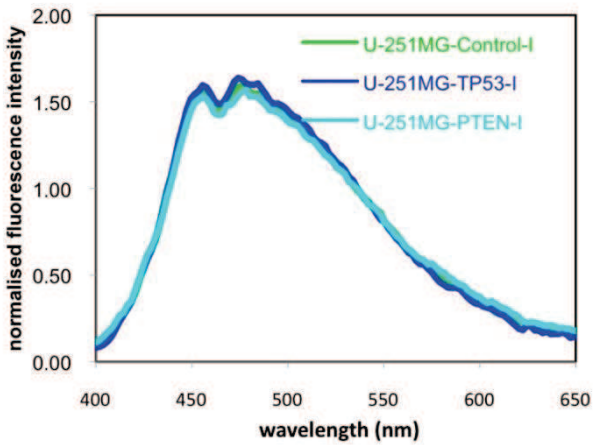
Data Aquisition and Analysis

Generation of autofluorescence images

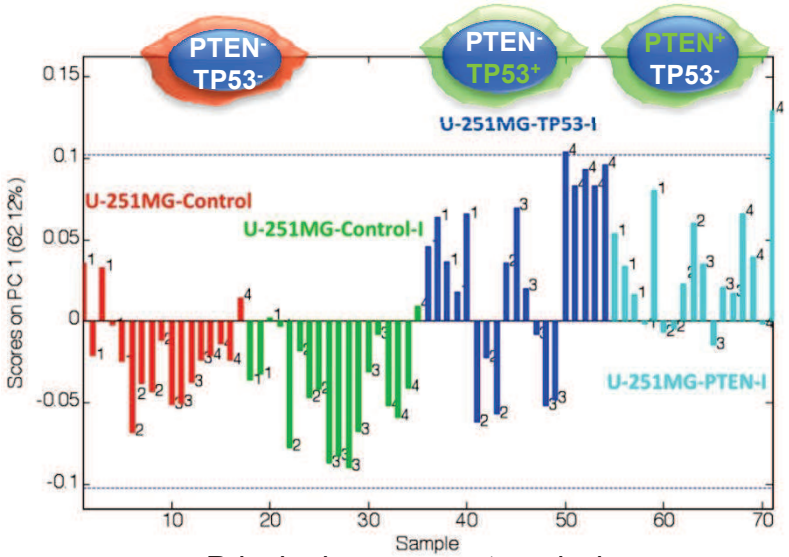


U251 MG monolayer

Multivariate data analysis



Mean normalised spectra (750 nm)

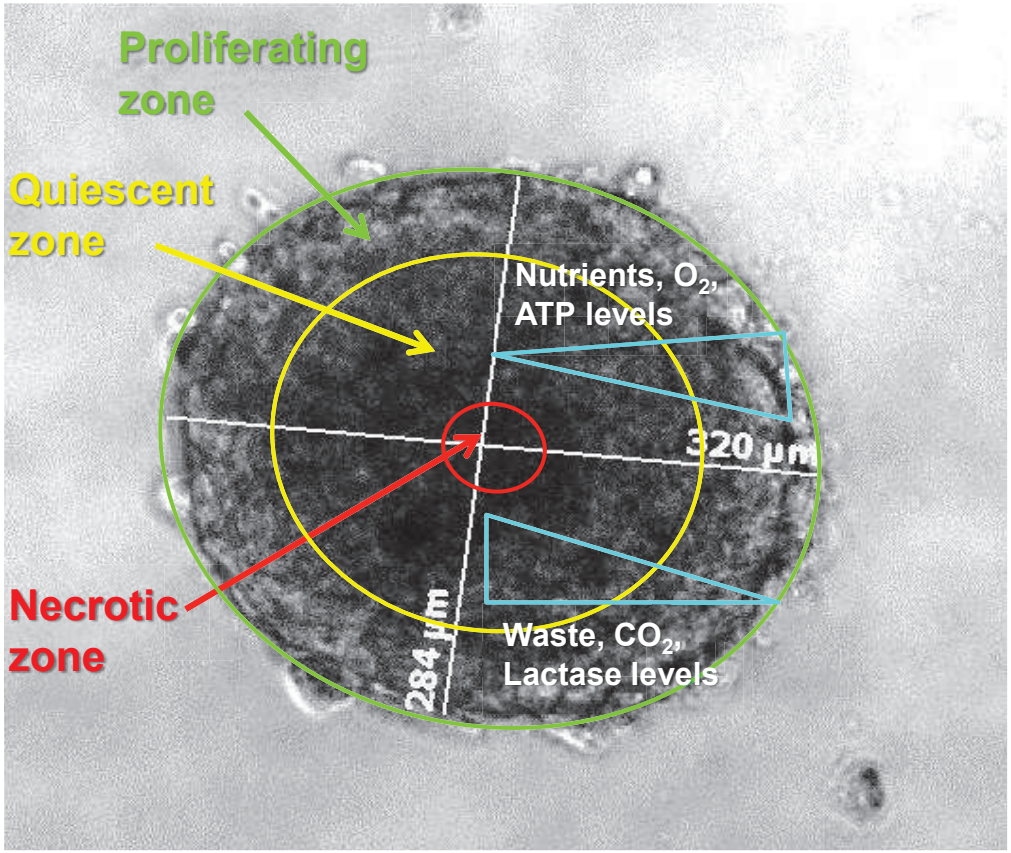


Principal component analysis

Holloschi, et al. 2012

II. Establishment of standardized multicellular U251-MG spheroid models (3D)

Multicellular Tumor Spheroids (MCTS)

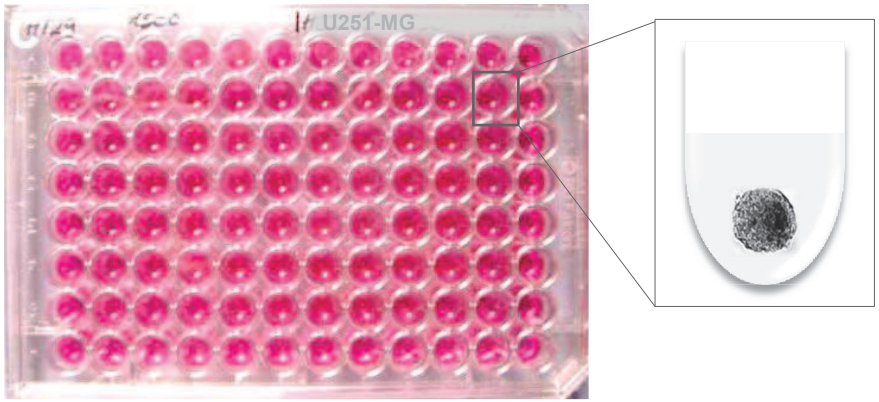


Spheroids at diameters between 200µm and 500 µm:

- resemble avascular tumor characteristics (micromilieu, volume growth kinetics, histomorphological features)
- reestablish morphological, functional and mass transport properties
- restoring an in vivo-like differentiation pattern due to the appropriate 3D extracellular matrix (ECM) assembly
- With increasing size, inward proliferation and oxygen/nutrient gradients are observed (pathophysiological gradients)
- potential accumulation of catabolites in central necrotic regions (poorly vascularized areas; > 500 µm)

Work Flow:
Standardized Spheroid Generation

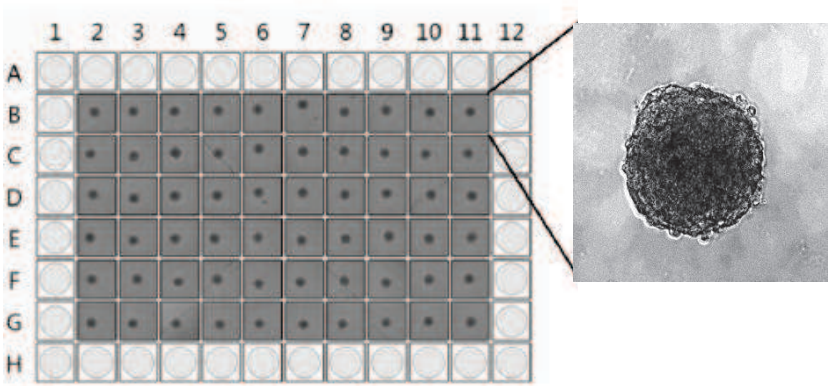
Cell seeding



ULA 96-well round-bottom plate
(Ultra-Low Attachment Surface)

- suspension culture
- single spheroid / well
- day 4: 300 – 400 µm (depends on cell type and cell number seeded)

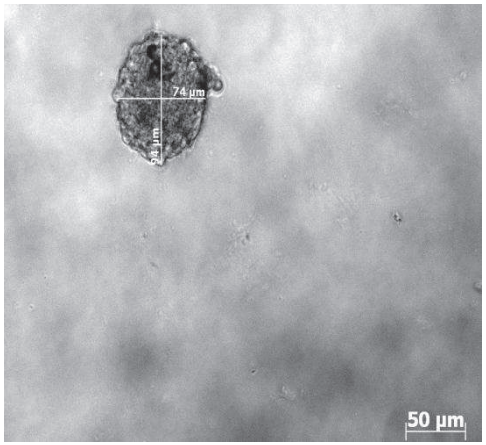
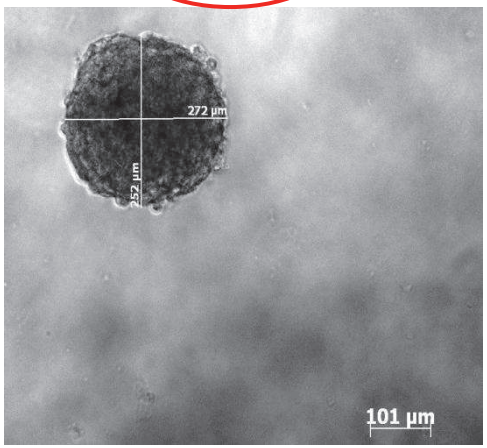
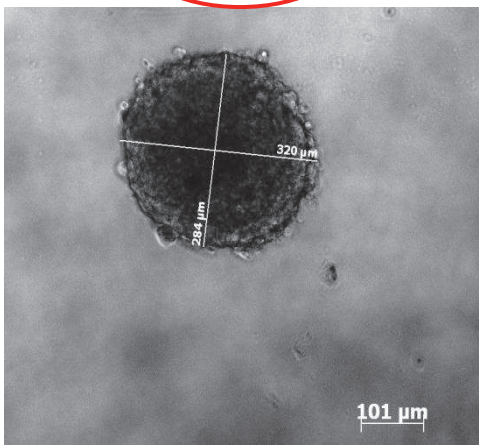
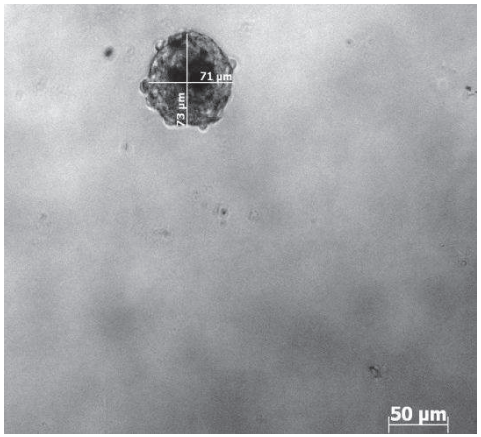
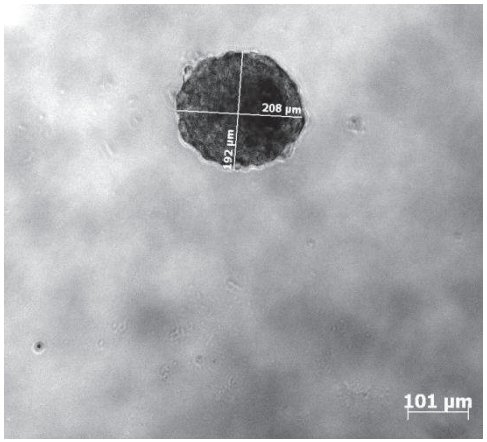
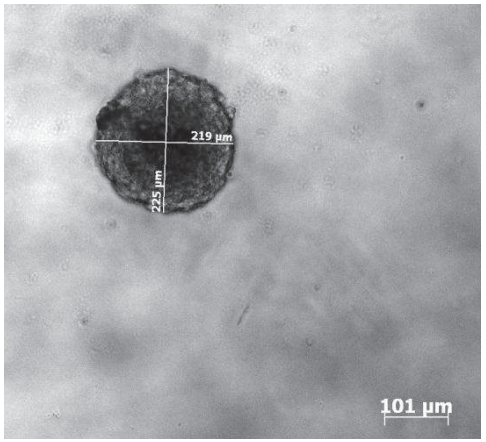
Imaging



- spheroid integrity (diameter; volume)
- reproducible size

Vinci, et al. 2012

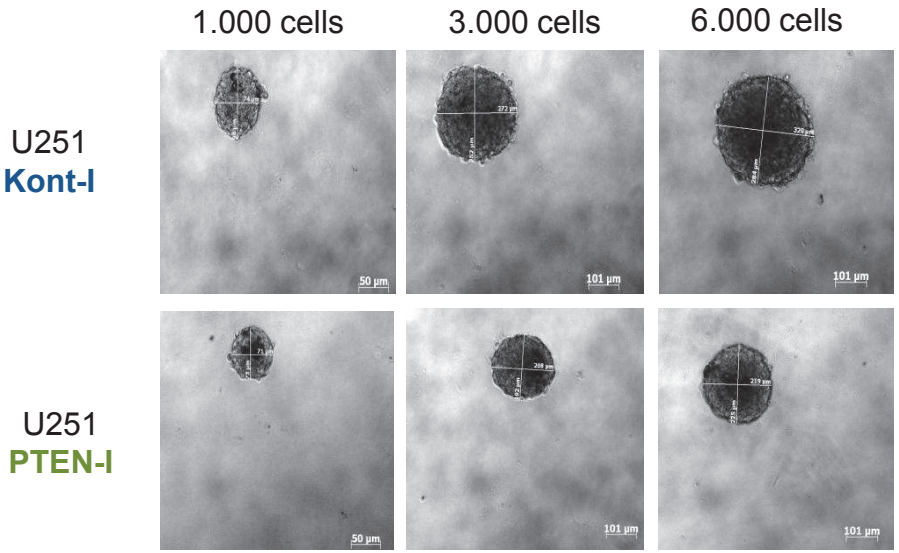
U251-MG: optimized cell seeding number reproducible spheroid size

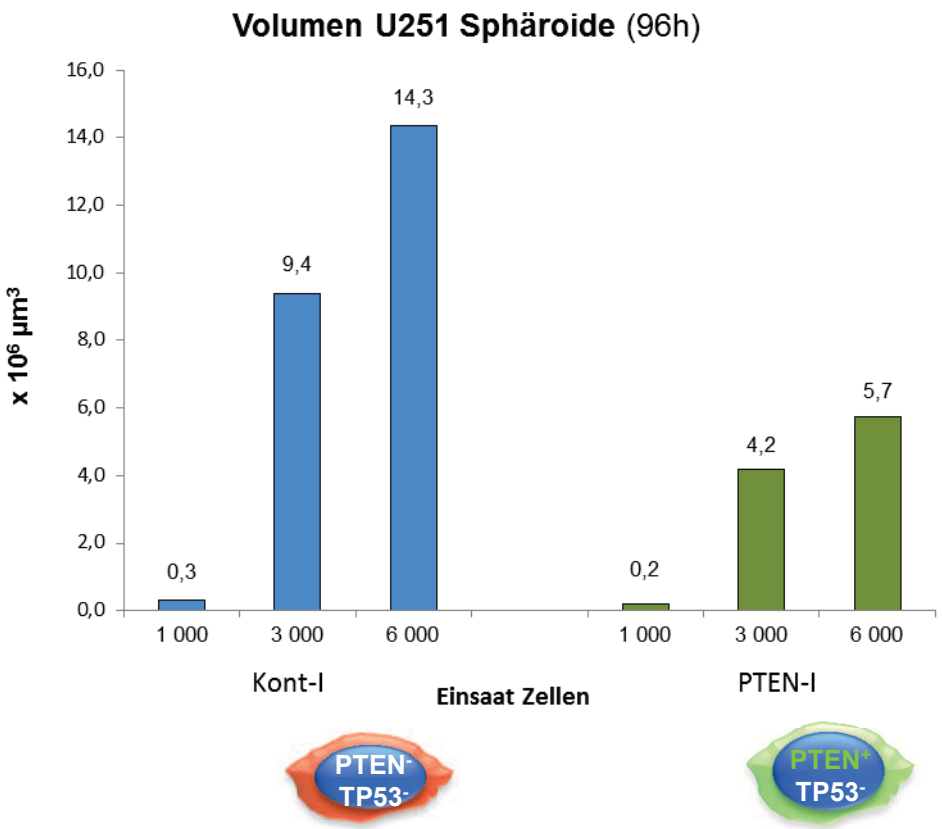
	1.000 cells	3.000 cells	6.000 cells
U251 **Kont-I** (PTEN⁻ TP53⁻)			
U251 **PTEN-I** (PTEN⁺ TP53⁻)			

96h after seeding; 10x

Analysis of spheroid size

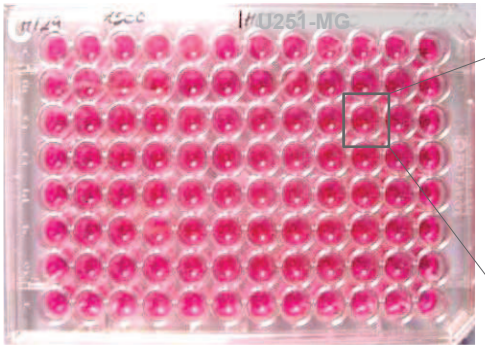
Influence of induced tumor suppressor PTEN to spheroid size in U251-MG cells

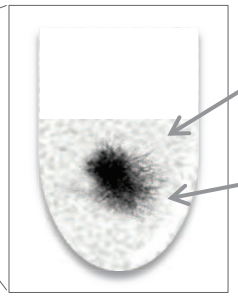




Spheroid Functional Assays: Invasion / Migration

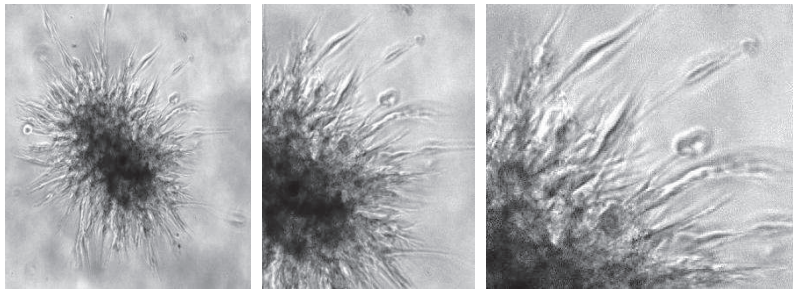
Tumor spheroid invasion





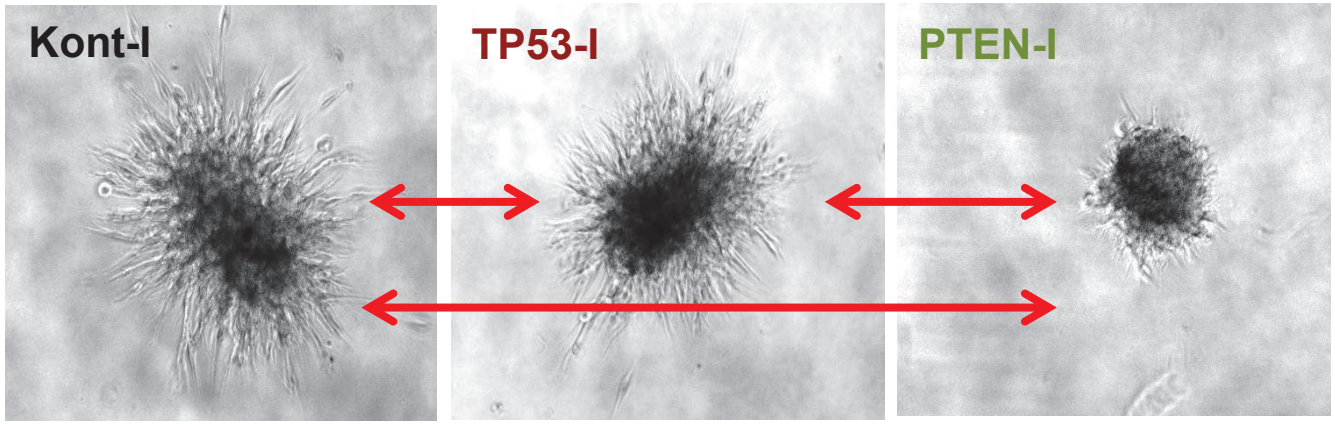
Invasion in Matrigel: 48h

Development of invadopodia (⟶ metastasis)



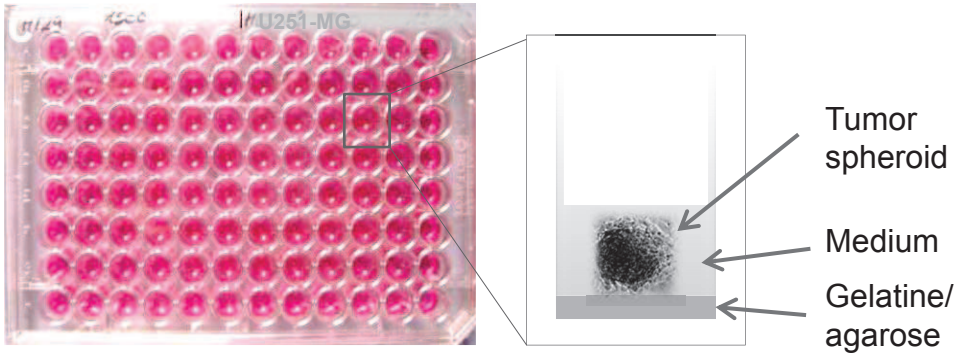
Measurements of invadopodia

Induced TP53 and PTEN show different effects to invasiveness of U251-MG cells

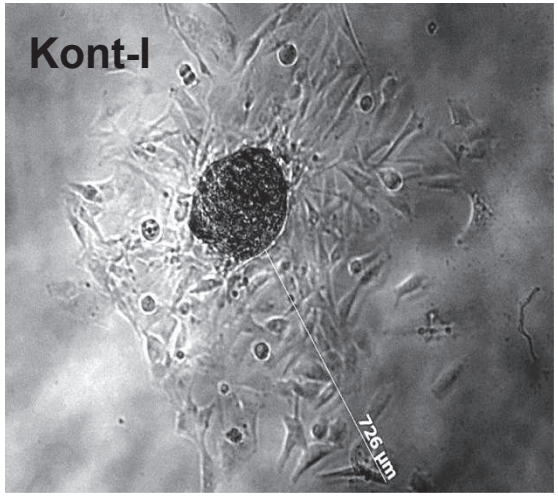


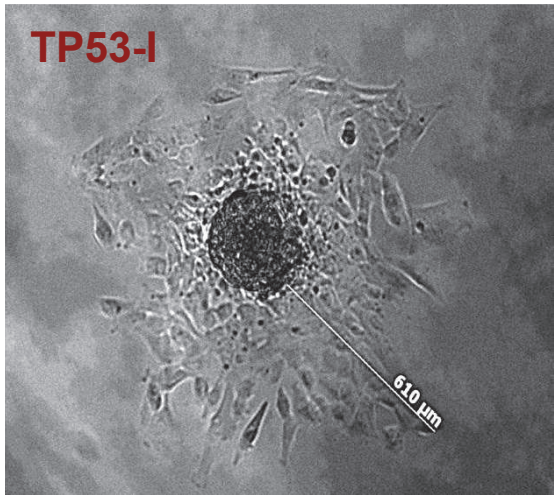
Spheroid Functional Assays: Invasion / Migration

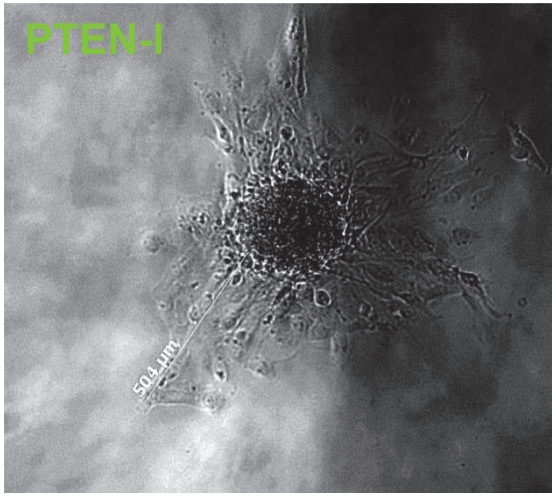
Tumor spheroid migration



➢ Induced TP53 and PTEN show different effects to migration and dissemination of U251-MG cells



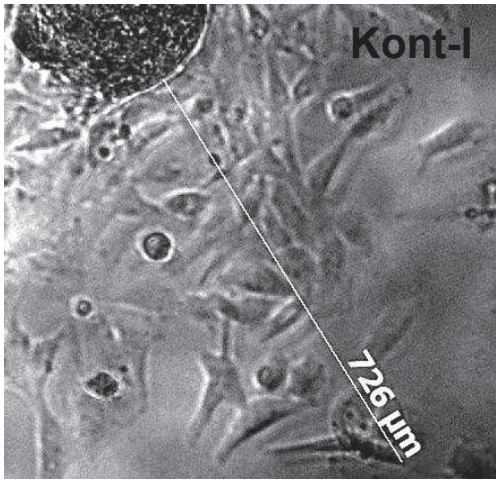




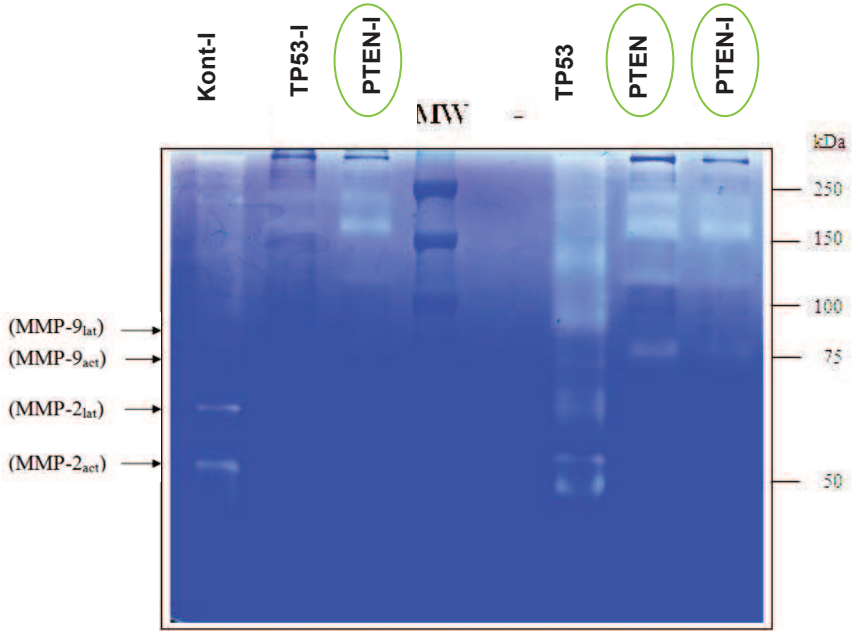
spheroids cultured on agarose (96 h), dissemination on agarose

Spheroid Functional Assays: Invasion / Migration

Tumor spheroid migration and dissemination



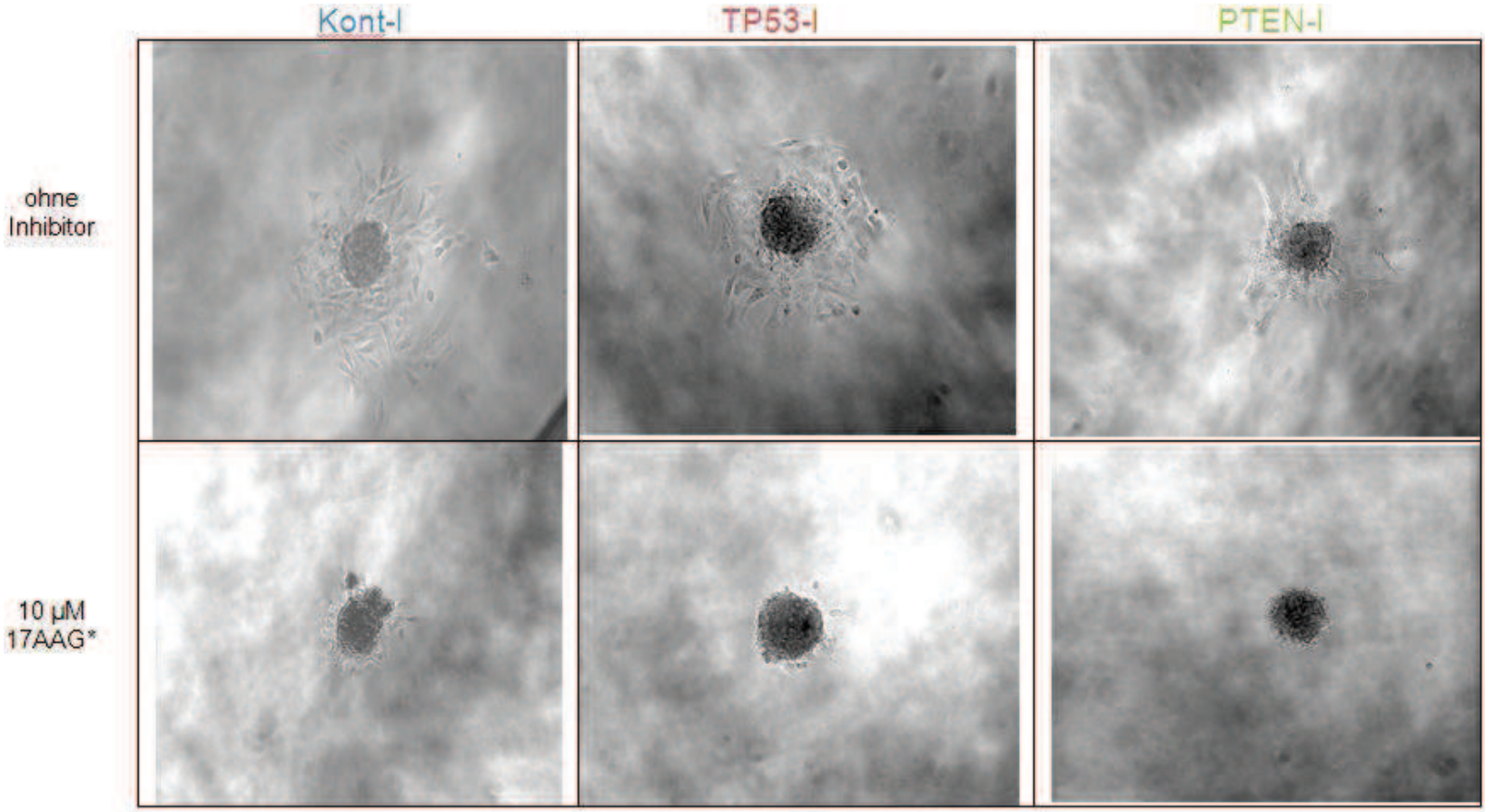
- **Zymogrames**; analytical tool for invasiveness



Matrix-metallo proteases (MMP)
- level is dependend on physiological conditions
- important in tumors for degradating ECM
- upregulated in tumors

Spheroid Functional Assays: Test of compounds

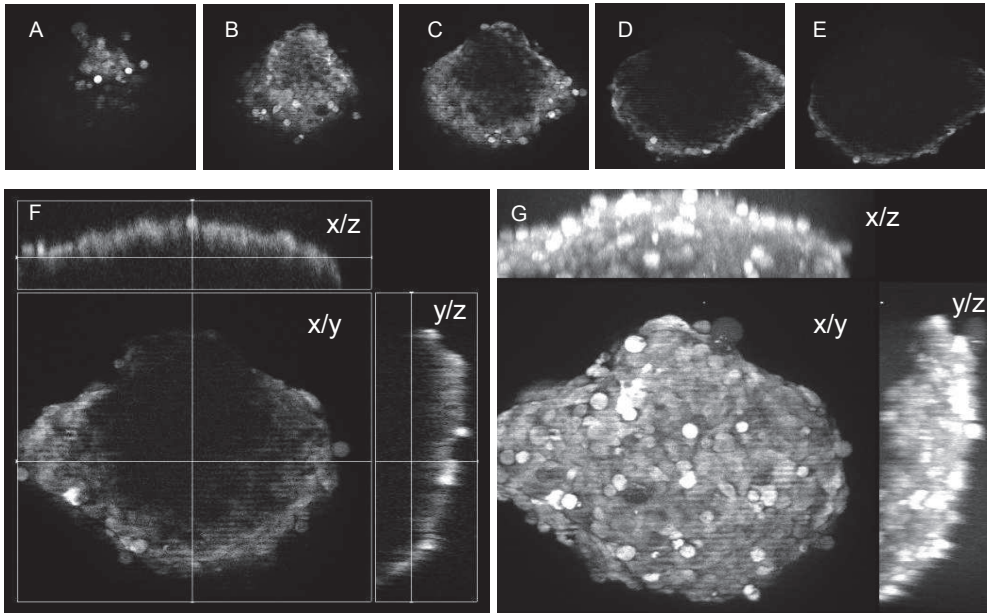
Influence of the HSP90 inhibitor 17-AAG to cell motility

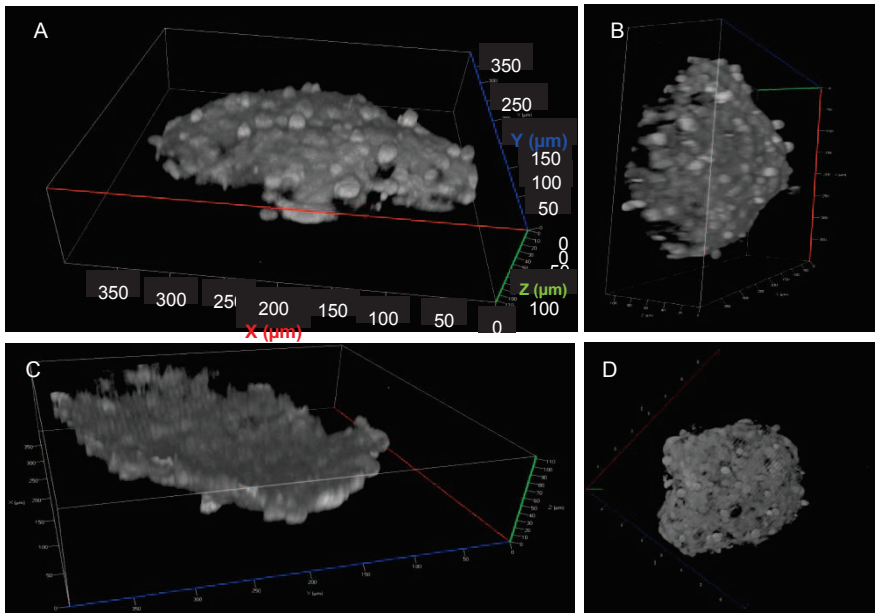


*17-N-Allylamino-17-demethoxygeldanamycin (hsp90-Inhibitor)

Microscopic analysis of spheroids

Analysis of U251-MG-Spheroids using ApoTome





2P Microscopy

Future directions

- **Spectral Imaging of isogenic tumor spheroids**
  - establishment of protocols for 2P-microscopy
  - analysis of 3D distribution of autofluorescence

- **Characterisation of spheroids**
  - analysis of biological parameters
  - analysis of structure

- **Transfer to other (cancer) cellular models (e.g. breast cancer)**
  - Validation of new target genes
  - 3D-cell models for ion channels

- **3D genetic and compound screens:**
  - expression of clinically relevant mutations
  - identifcation of new tumor candidate genes
  - testing new potential drug candidates

- **Production of spheroids in micro-bioreactors**
  - standardized, parallel production in controlled processes

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ZAFH-Photon^{n}

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