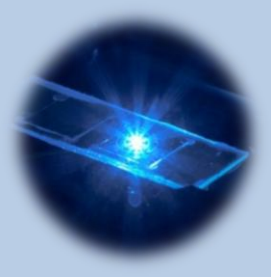
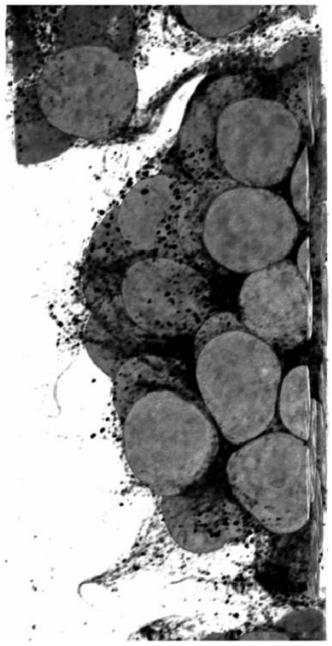




3-D model systems for

CANCER BIOLOGY

VTT offers a miniaturized, three-dimensional screening platform for small compound testing and drug/drug target validation. Standardized experimental conditions have been optimized for rapid drug screening and high-content microscopic imaging. Real-time, live cell microscopy and automated image analysis software solutions provide adequate tools to monitor the dynamics and heterogeneity of cell growth, differentiation, invasion and other highly relevant morphologic features.

Compared to monolayer cell culture, 3D model systems more accurately mimic the complexity of breast, ovarian and prostate cancer biology, and provide relevant answers related to cancer treatment and drug development. 3D models represent a valid intermediate validation step between primary drug screening and animal experimentation.

Readout
- Phase Contrast Microscopy
- Live Cell Imaging
- Confocal Microscopy
- Immunofluorescence
- Biomarker Expression
- mRNA and Protein Expression

All-in-One Assays
- Proliferation
- Apoptosis and Cell Death
- Growth Dynamics
- Cell Differentiation & Morphogenesis
- Cancer Invasion

Applications
- Compound Screening & Drug discovery
- Target Validation
- Toxicology
- Stem cell research
- Basic Cell Biology
- Co-Culture Models

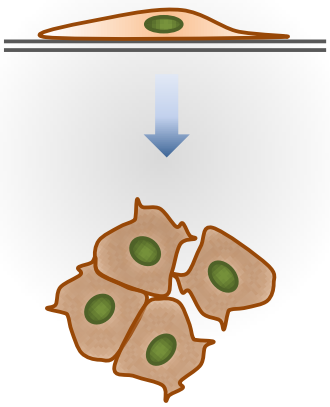
Additional information

Matthias Nees
Senior research scientist
Tel. +358 40 8314839
matthias.nees@vtt.fi

2-D versus 3-D cell culture

2-D monolayer culture

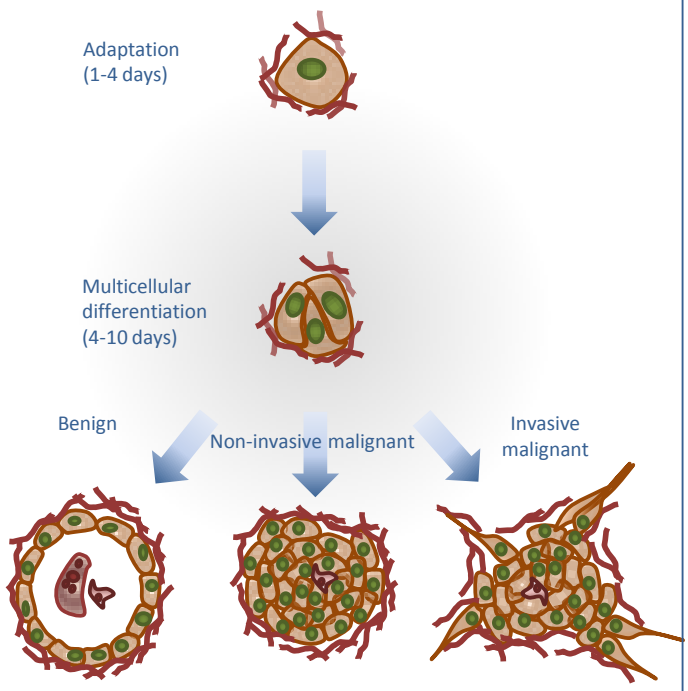
Cells plated on coated or uncoated plastic surfaces



Monolayer culture
- Highly reductionist
- Artificialilty
- Weak external stimuli
- Limited differentiation potential
- Motility and invasion mechanisms poorly represent real clinical tumors
- Over-emphasized proliferation machinery

3-D organotypic culture (glandular epithelium)

Cells embedded in ECM substrate

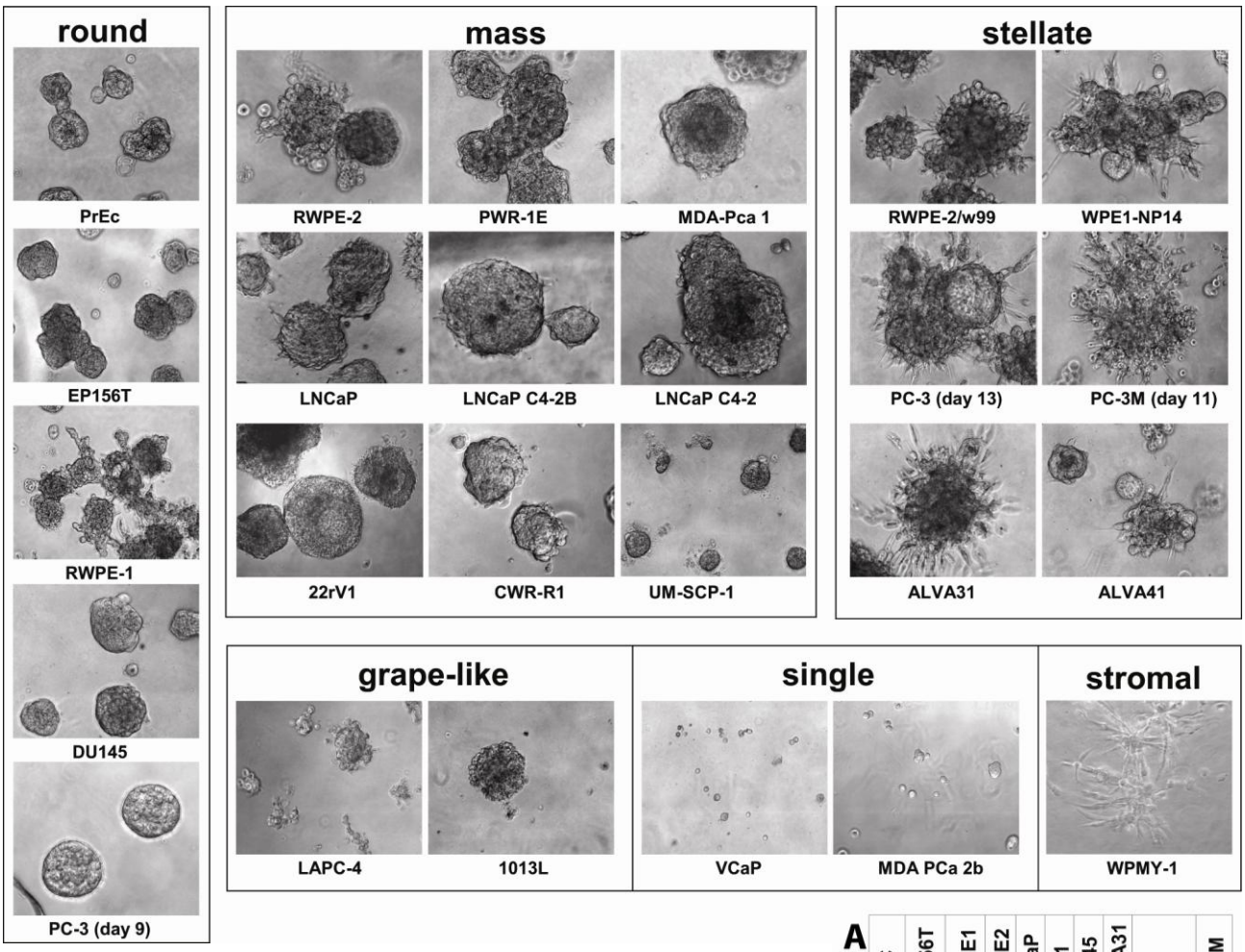


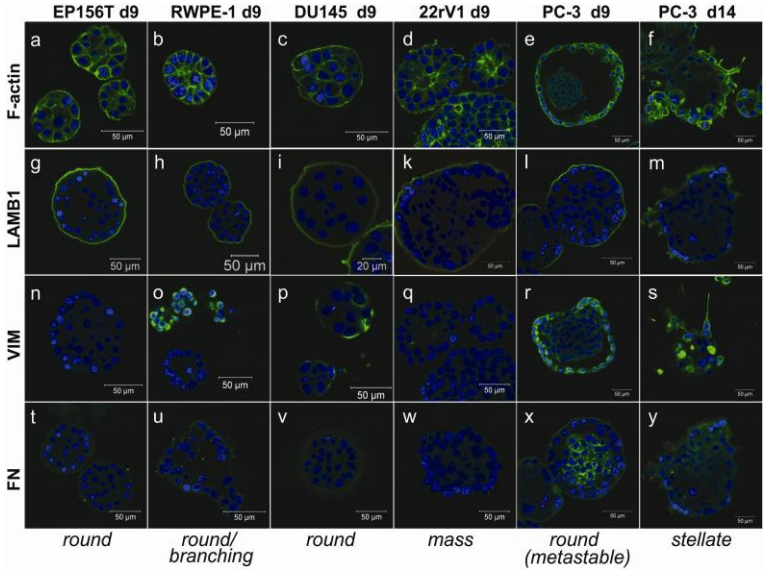
Acini
- Highly organized and polarized multicellular epithelial structure
- Well developed basal lamina
- Hollow lumen

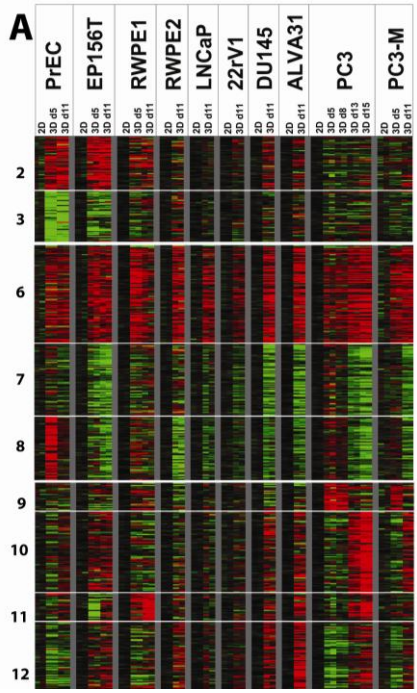
Cell masses
- Poorly organized structure
- Incomplete or lacking basal lamina
- Apoptotic cells/hypoxia in the core

Invasive
- Cells projecting into the surrounding ECM
- Active ECM degradation

ALL-IN-ONE ASSAYS



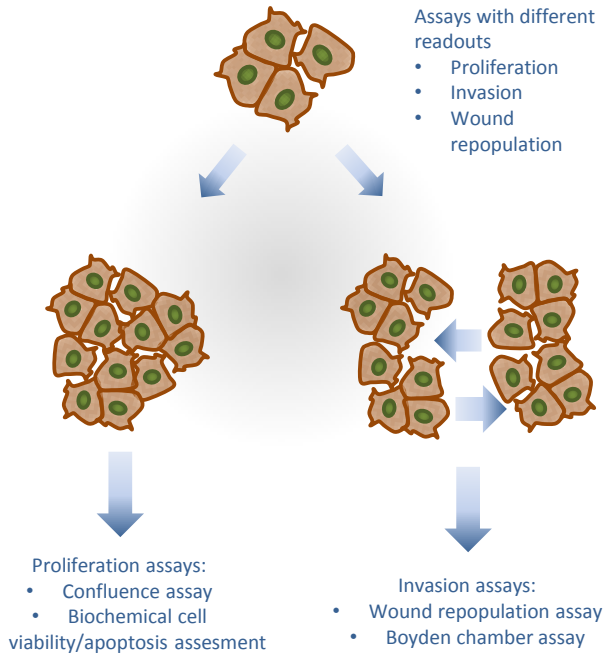




Comprehensive Panel of Three-Dimensional Models for Studies of Prostate Cancer Growth, Invasion and Drug Responses. Härmä V, Virtanen J, Mäkelä R, Happonen A, Mpindi J-P, et al. 2010 A Comprehensive Panel of Three-Dimensional Models for Studies of Prostate Cancer Growth, Invasion and Drug Responses. PLoS ONE 5(5): e10431. doi:10.1371/journal.pone.0010431

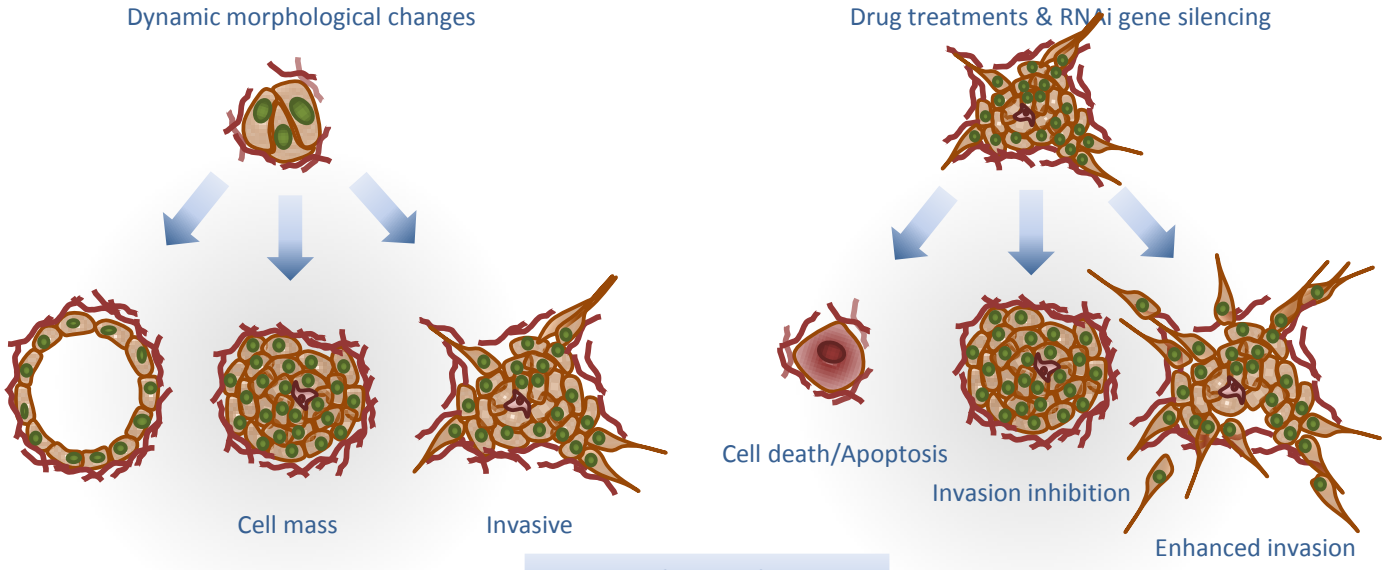
2-D versus 3-D assays

2-D assay examples



Assays usually mutually exclusive

3-D assay examples



Single readout

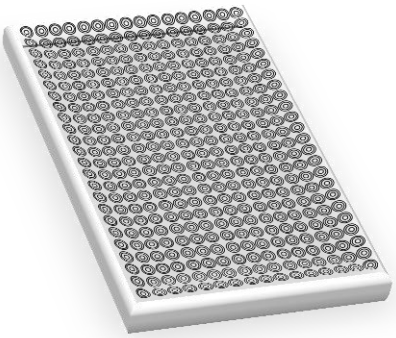
All-in-one assays with one single readout
- Multicellular growth
- Cell death & apoptosis
- Morphological differentiation
- Invasion

3-D model systems for

CANCER BIOLOGY

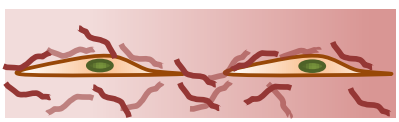
3-D structures positioning

High-throughput/high-content multiwell plates for miniaturized 3-D cell cultures



Cells attach to a smooth layer of solid extra-cellular matrix

Cells are covered with another layer of matrix



Multicellular differentitation

Multicellular structures growing in one single optical plane

VTT innovations enable cost-effective and reproducible 3-D assays

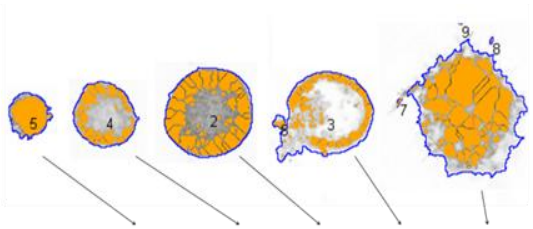
Miniaturization

Traditionally, 3-D culture is considered tedious, time consuming, and expensive, based on the need for specialized cell culture media and costly biological materials that support 3-D growth. Thus, a high level of miniaturization and automation, and affordable plastic consumables, are key for cost-effective assay design. We have reduced the volume of a single experiment to 10 – 25 µl, cutting the material cost to approx. 1 €/well. These overall costs can be further reduced based on our well-in-a-well design, which allow a high level of miniaturization (96 vs. 384 well plate format), and reduce researcher intervention. Such 3-D cell culture materials will ideally match the technical and geometrical requirements of established lab automation equipment, including cell culture robots. Furthermore, lab automation may not interfere with the overall optical quality to warrant automated, high-content microscopy. As the task leaders in this field, VTT-MBT has 8 years of experience in high throughput screening technologies, lab automation, microscopic techniques, and process optimization.

State-of-the-art image analysis software

The biological relevance of functional screens depends on robust information that can be concluded from the most informative and specific assays. At VTT-MBT, we currently combine high content confocal and phase contrast microscopy with automated image analyses, based on in-house software solutions. Automated image analysis relies on morphological parameters related to size, shape, differentiation, and invasive properties of 3-D structures. Dynamic morphological changes, e.g. in response to small inhibitor treatment, siRNAs, or stress conditions, are thus statistically evaluated and quantified. Apoptosis and cell proliferation are readily evaluated based on live-cell staining with reactive dyes, and can be similarly automated. The existing concept of smart cell culture consumables facilitates auto-focusing of microscopic devices on cell structures, exclusion of blurring “meniscus effects”, and imaging of as many as possible structures in parallel. Only under these conditions, automated image analysis tools will be capable to statistically evaluate large amounts of images from high-throughput screens.

3-D image analysis



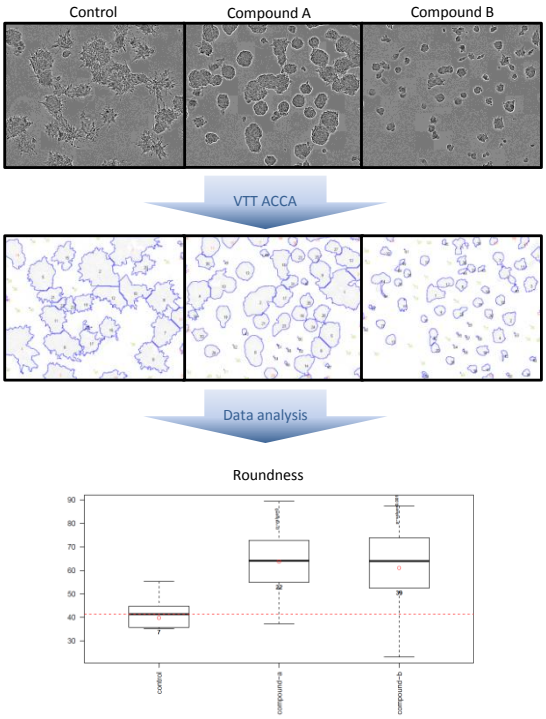
Area	1301	2833	5558	5469	9209
Roundness	77.70	81.61	88.24	46.67	67.28
RoundDiff	5.69	2.10	-0.22	4.55	4.36
AppIndex	0.65	0.33	0.14	1.36	1.64
Roughness	1.92	0.81	0.22	1.55	4.01
Density	133.85	78.70	129.82	47.95	104.21
AppNumber	0	0	0	5	4
Hollowness	7.24	78.64	76.75	77.78	53.57

VTT has developed a specialized software to quantitate various key morphogenetic parameters related to multicellular differentiation, growth and invasion.

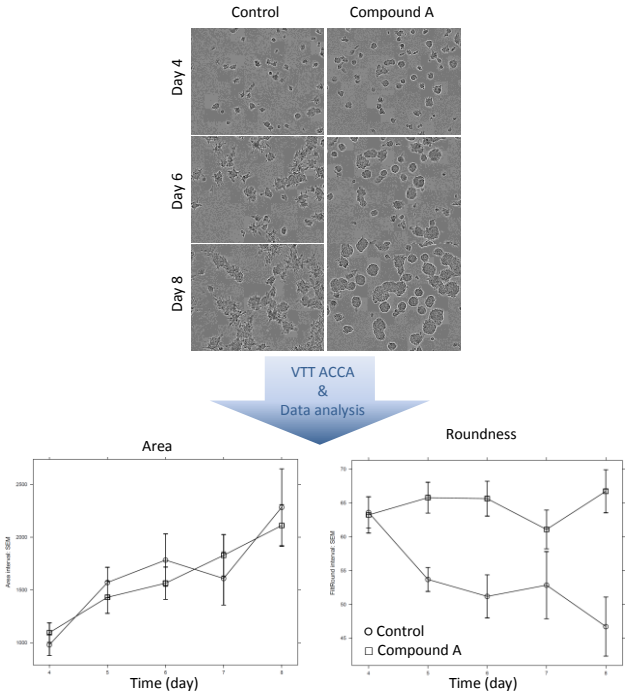
VTT ACCA – software solutions for 3-D cultures

Phase contrast image analysis

Endpoint

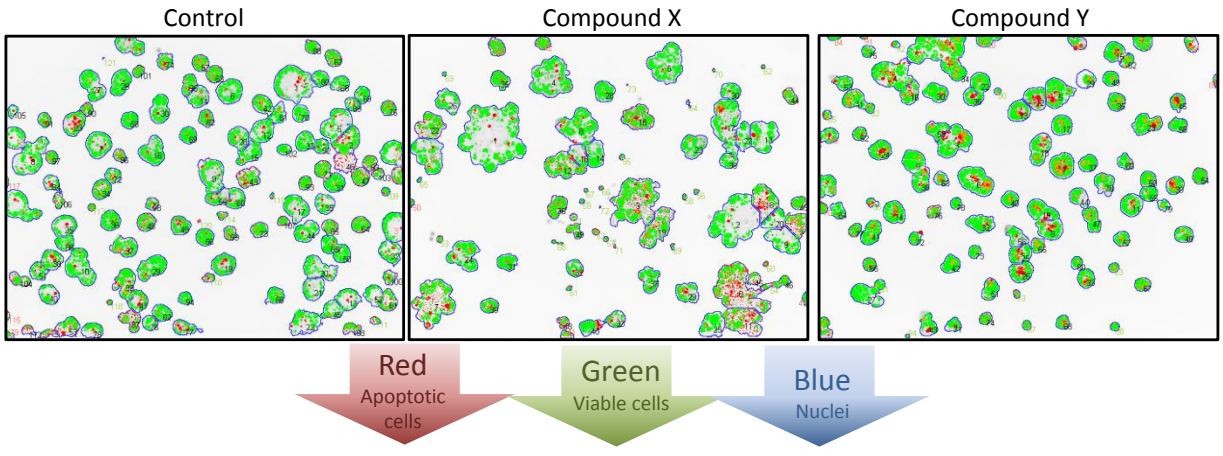


Time-lapse



VTT ACCA readily analyzes phase-contrast images acquired with any standard laboratory microscope. Analyses can be made from endpoint or time lapse experiments.

Fluorescence-based and 3-D stack image analysis



VTT ACCA analyzes multichannel images acquired with standard fluorescence microscope or confocal systems. Images can be single intensity projections or 3-D stack images. To increase assay flexibility each channel can be analyzed separately.

3-D model systems for

CANCER BIOLOGY

At glance: High Content 3-D Model Systems for Cancer Drug Discovery

Cell Lines

- Models specifically addressing the most relevant problems in oncology: invasive growth and metastasis, proliferation, and cell death (full spectrum of tumor biology)

Miniaturization

- Miniaturized, high-content screening platform
    - Standardized, reproducible, cost-effective, flexible

Microscopic Imaging

- Real-time monitoring of complex organotypic cell cultures for up to 30 days
- Accurate measurements of dynamic cellular features without cell fixation
- Confocal/Fluorescence microscopy allows simultaneous measurement of multiple relevant endpoints such as biomarker expression, apoptosis, and substructural localization.

Automated Image Analysis

- VTT software solutions address the specific demands of high-content screening
    - Acquisition, storage, automated analysis, statistical evaluation and quantification
- Informative single-readout live cell assays (e.g. for apoptosis, proliferation, invasion) are available.

Drug Response

- Long term exposure e.g. for toxicology and cell differentiation studies
- Drugs that fail in 2D may be effective in 3D. This adds a very relevant layer of information for cancer drug discovery and cell biology

Molecular Biology and Bioinformatics

- Supporting quantitative biochemical methods that complement our phenotypic 3D assays have been established
    - Protein, mRNA and miRNA profiling, reversed-phase protein arrays, and quantitative realtime RT-PCR

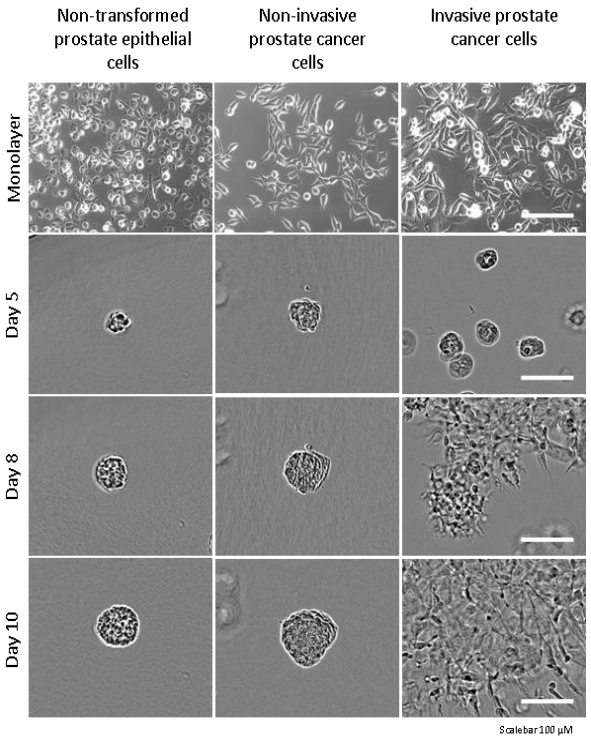


Figure 1. Prostate epithelial cells from different stages of cancer have distinct morphologies in 3-D organotypic culture.

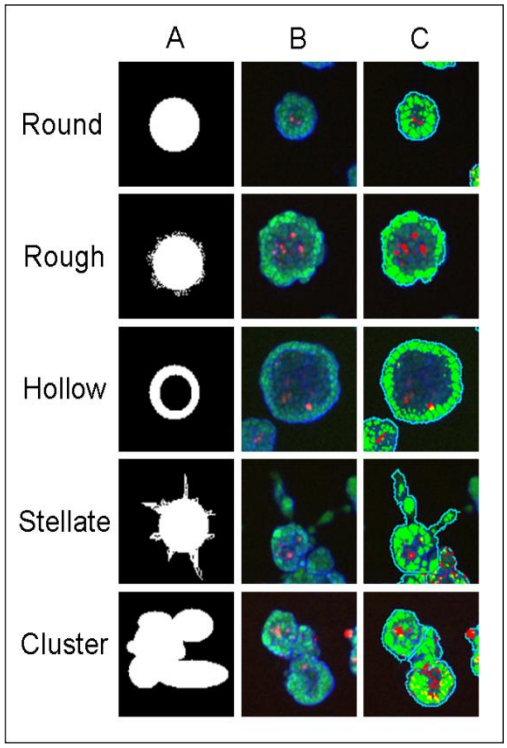


Figure 2. VTT ACCA is an automated image analysis software specialized for high-content and high-throughput quantification of several key morphological parameters related to multicellular differentitation, growth and invasion.