ImageStreamX, Raman Spectroscopy & Multiphoton Imaging for Cell and Tissue Monitoring

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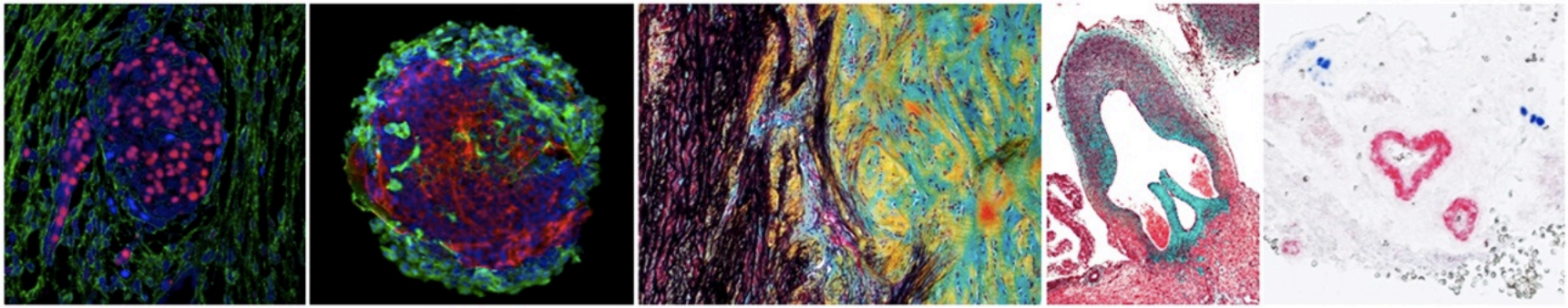
Tübingen, July 22nd 2014



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ImageStream^{X} mk II

ImageStream^{X} mk II

A combination of Fluorescence Microscopy and Flow Cytometry

- Next generation instrument that overcomes limitations of both techniques

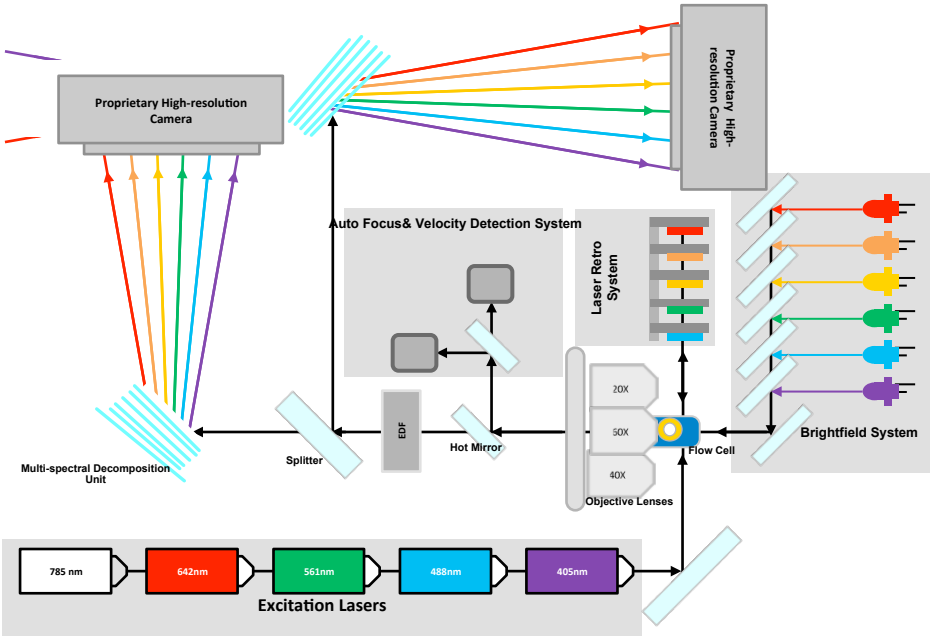
- ImageStream^{x} in Germany: 7 machines only
  so far no Core Facility

First ImageStream^{x} - **Core Facility**

ImageStream^{X} mk II

Specifications

- 5 lasers: standard 488nm, 405nm, 561nm, 642nm and SSC (785nm)
- 12 image channel
- MultiMag 20x, 40x and 60x
- Extended Depth of Field (EDF^{TM})
- 2 CCD Cameras

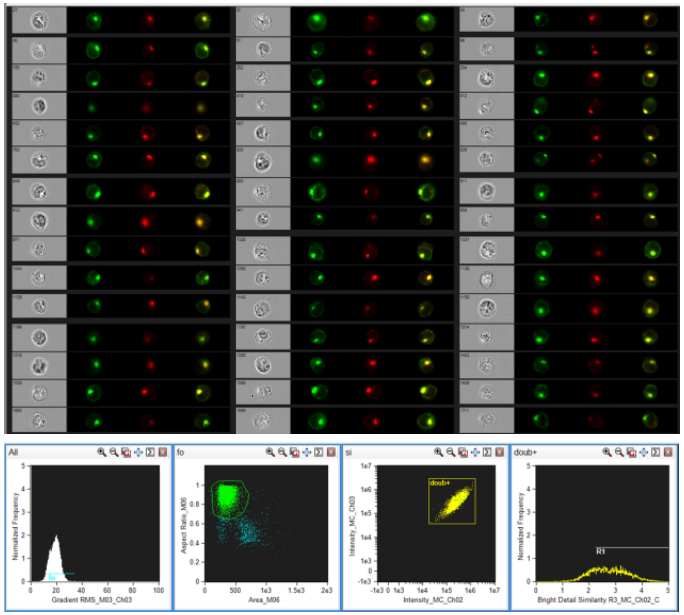


ImageStream^X mk II

Faster: Aquisition of up to 4000 cells/sec

Easier: Real-time plotting and graphical gating plus imaging of every cell; easy-to-use-compensation wizard

High Efficiency: Up to 95% of sample -- unused samples can be recovered



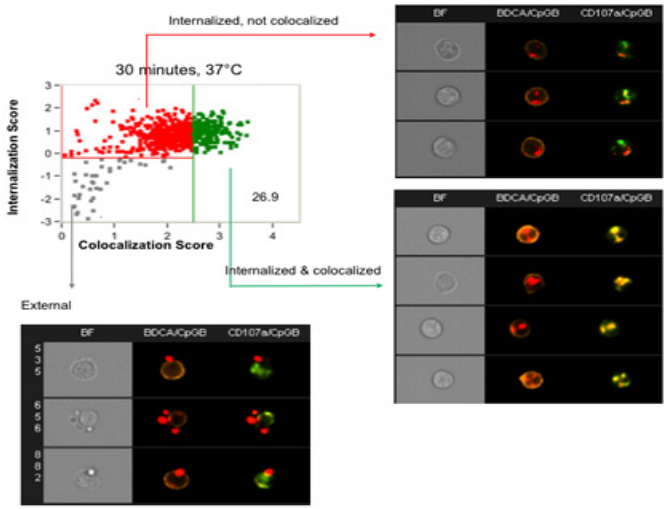
ImageStream^{X} mk II

- Combines speed, sensitivity and phenotyping abilities of flow cytometry and high-resolution microscopy
- High-resolution images of single cells in flow
- Unique combination ⟹ opens door to wealth of applications:

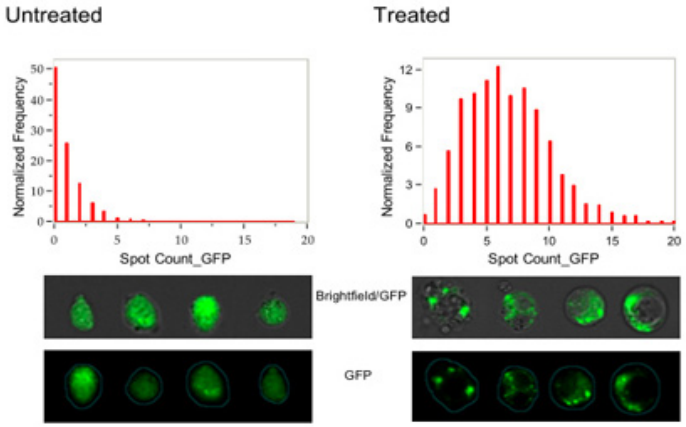
Cell Signaling	DNA Damage and Repair
Co-localization	Cell Cycle and Mitosis
Cell-cell interactions	Autophagy
Morphology	Stem Cell Differentiation
Internalization	Cell Death

ImageStream^X mk II -- Applications

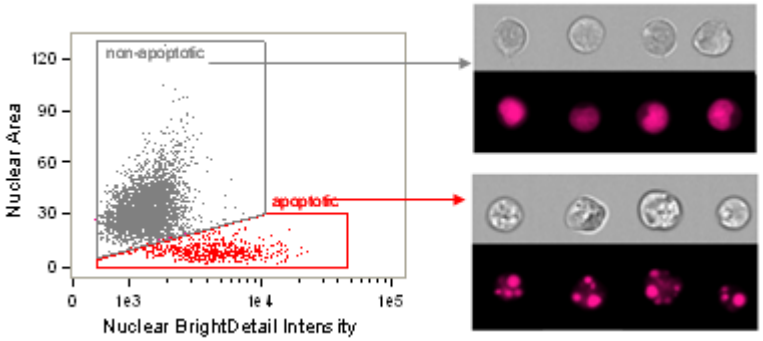
Co-localization



Autophagy

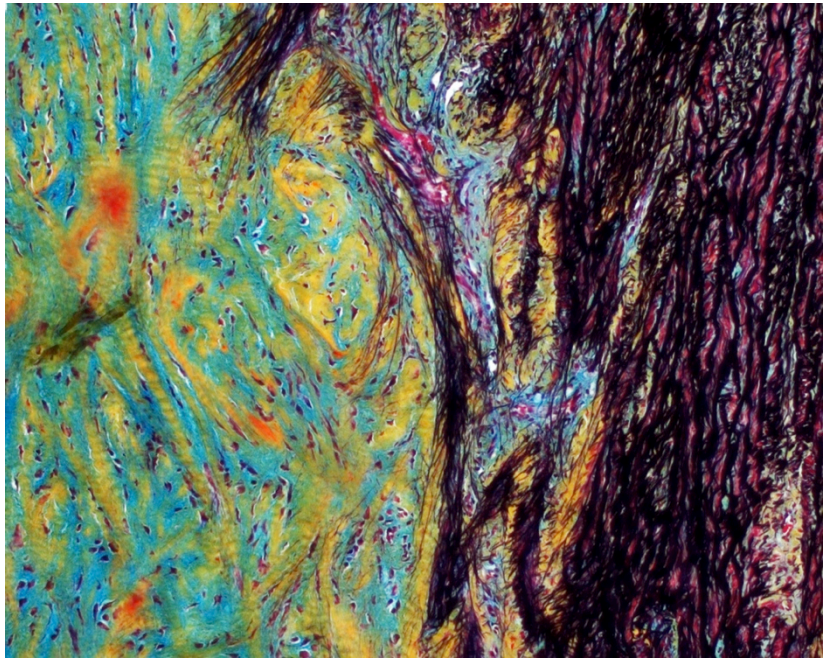
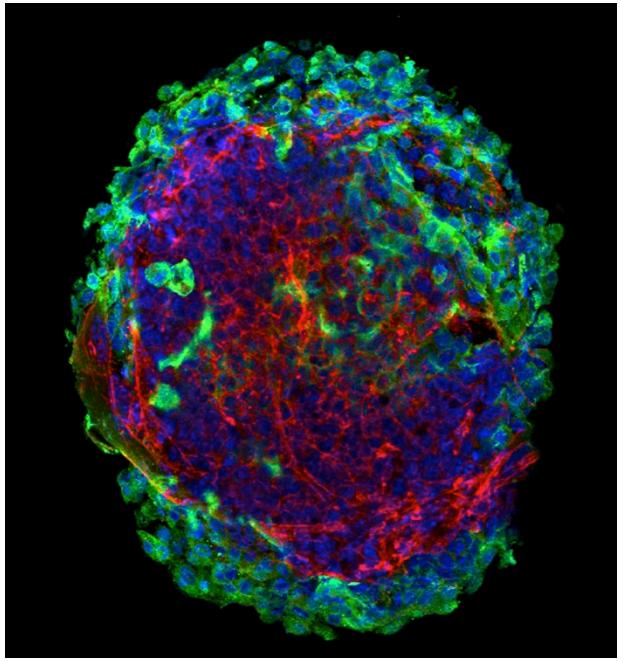
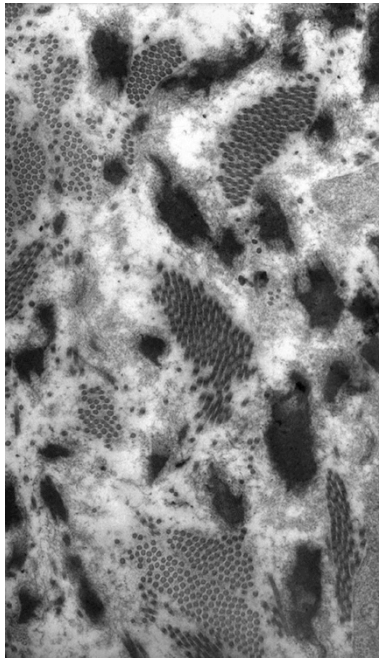


Apoptosis



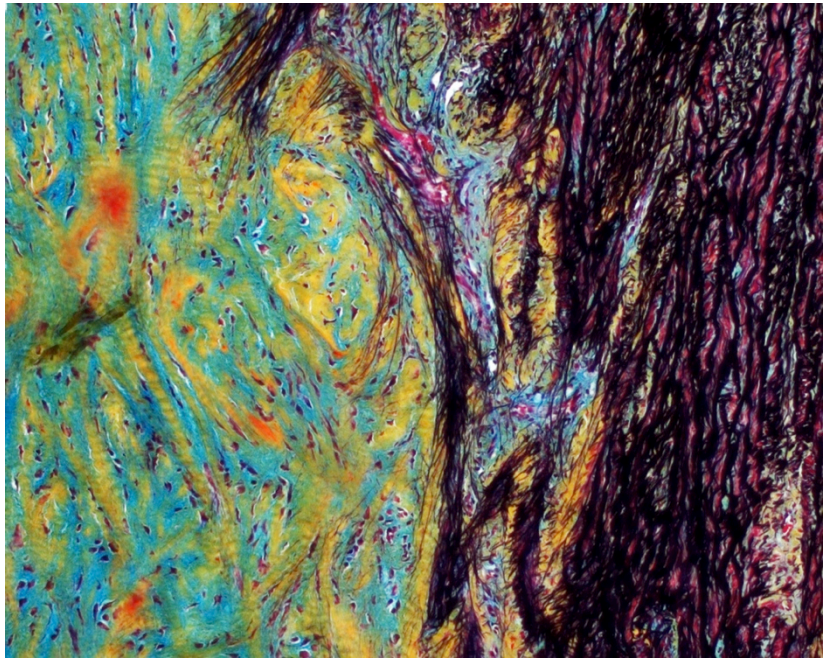
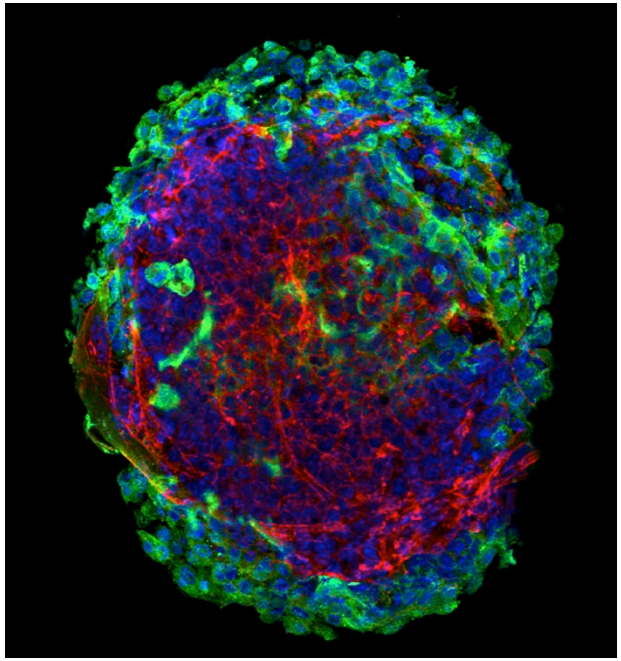
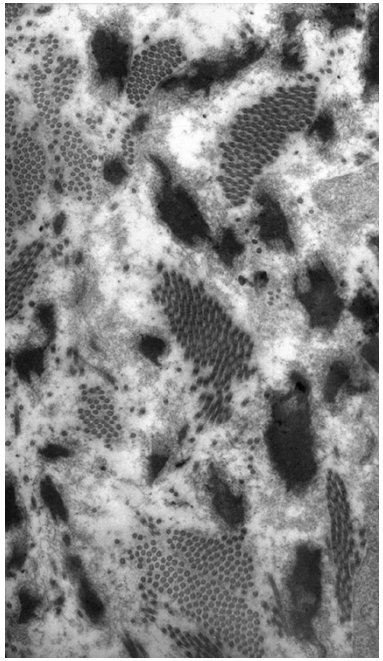
Non-invasive monitoring of cells & tissues

- Multiphoton imaging
- Raman spectroscopy

Non-invasive monitoring of cells & tissues

- **Multiphoton imaging**
- Raman spectroscopy

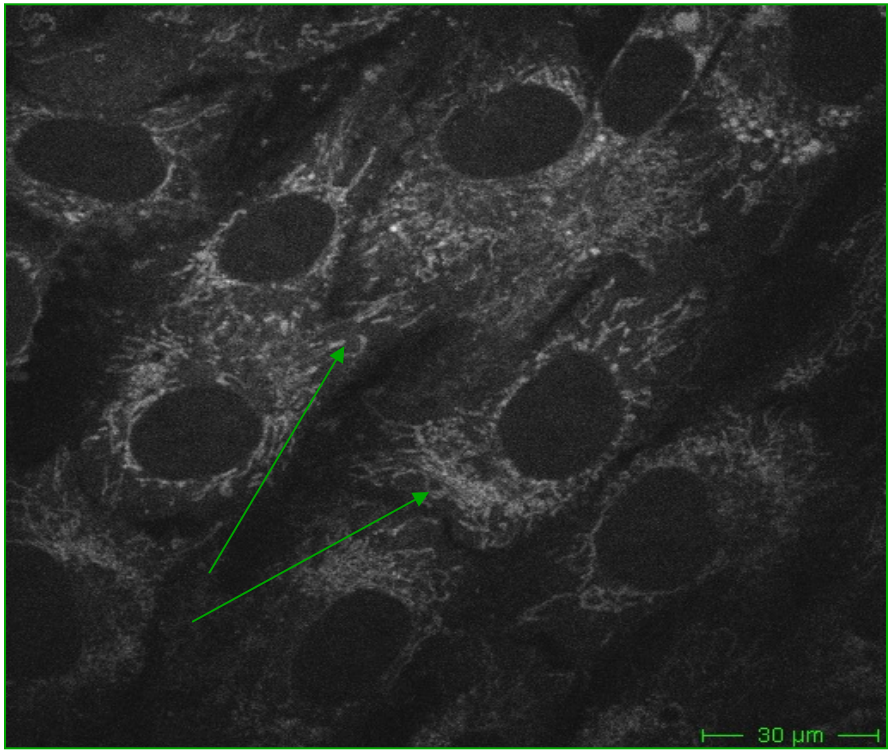




Multiphoton imaging

- detect endogenous and exogenous fluorophores

Endogenous fluorophores

- NAD(P)H
- Flavines
- Melanin
- Thyrosin (UV)
- Tryptophan (UV)
- Porphyrins
- **Elastin**
- **Collagen (SHG)**



Mitochondrial network (NAD(P)H) of human pancreatic stem cells. Green arrows show single mitochondria; ex: 750 nm, em: 450-470 nm

Multiphoton imaging

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Endogenous fluorophores

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- **Elastin**
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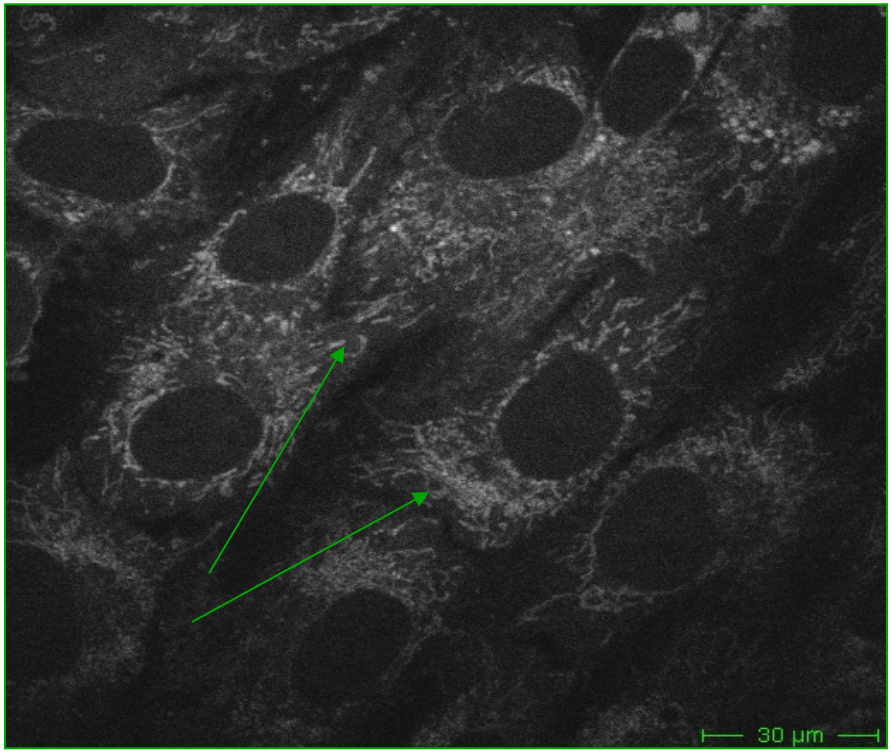
No staining!
No fixation!



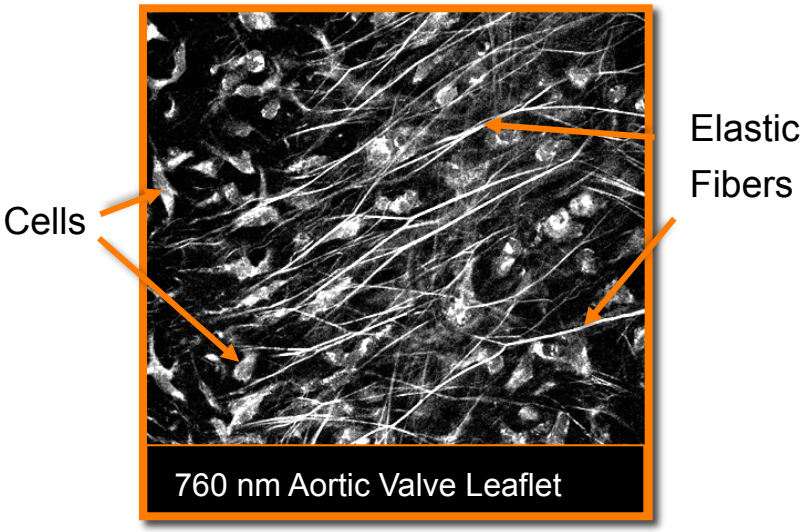
Mitochondrial network (NAD(P)H) of human pancreatic stem cells. Green arrows show single mitochondria; ex: 750 nm, em: 450-470 nm

Non-invasive monitoring of cells & ECM structures

Excitation Wavelengths:

740-760 nm

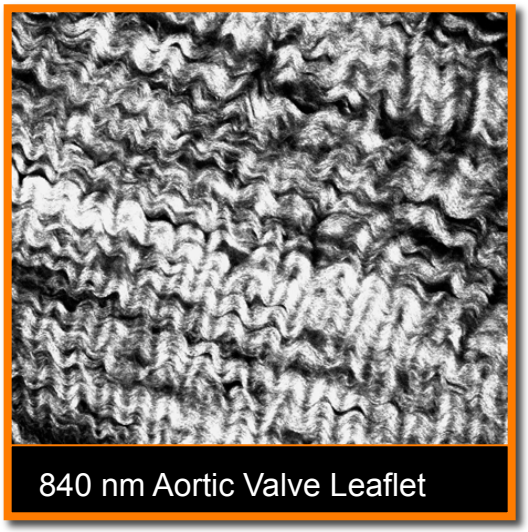
Elastic Fibers



Excitation Wavelengths:

840-860 nm

Collagen Fibers



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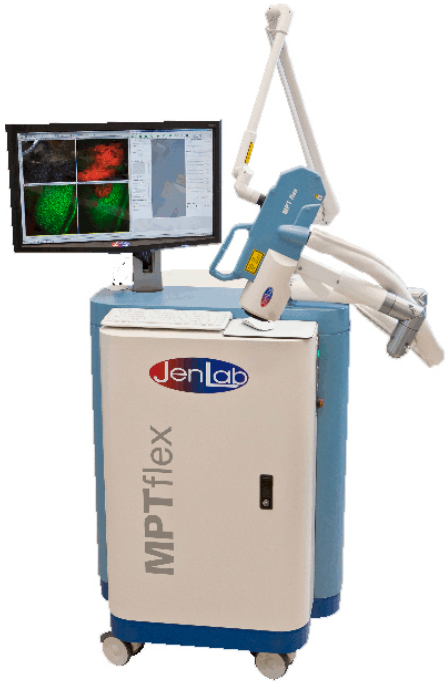
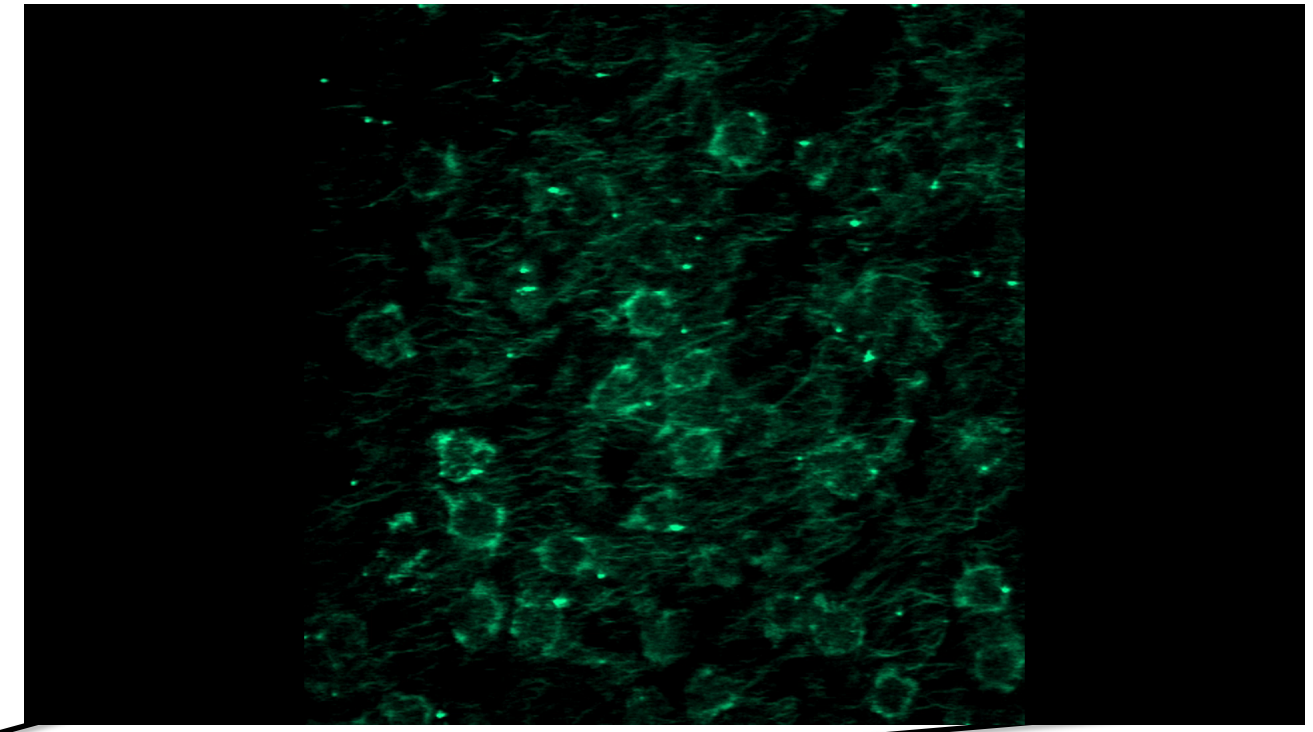
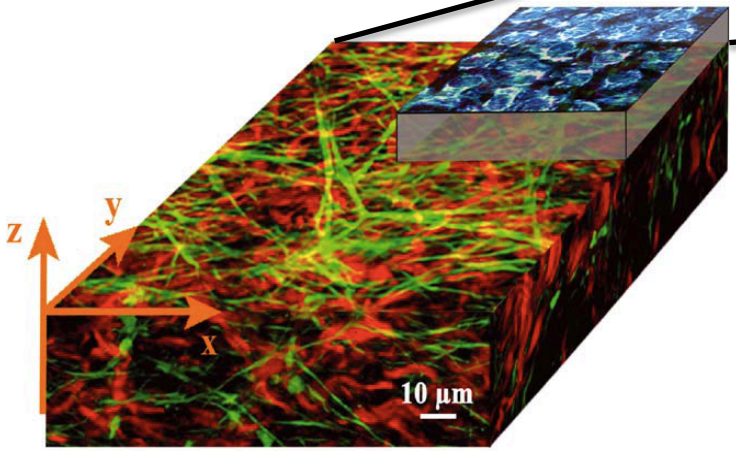
Brockbank KG et al.
Cells Tissues Organs 2011

Brockbank KG et al.
Ann Thorac Surg 2011

Pre-implantation quality control



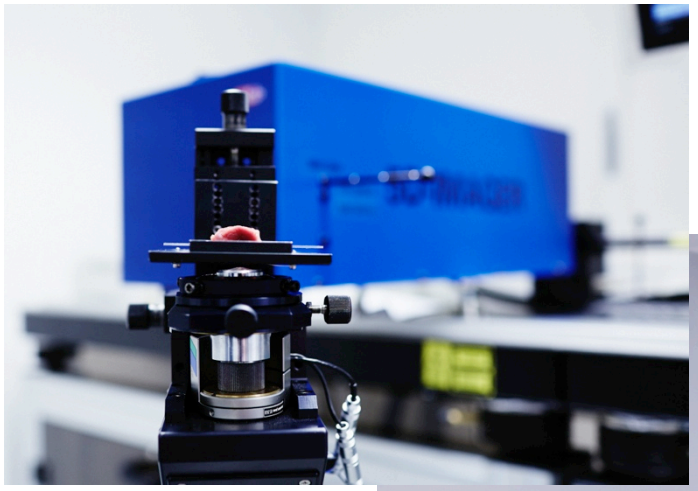




König K et al. *Biomaterials* 2005

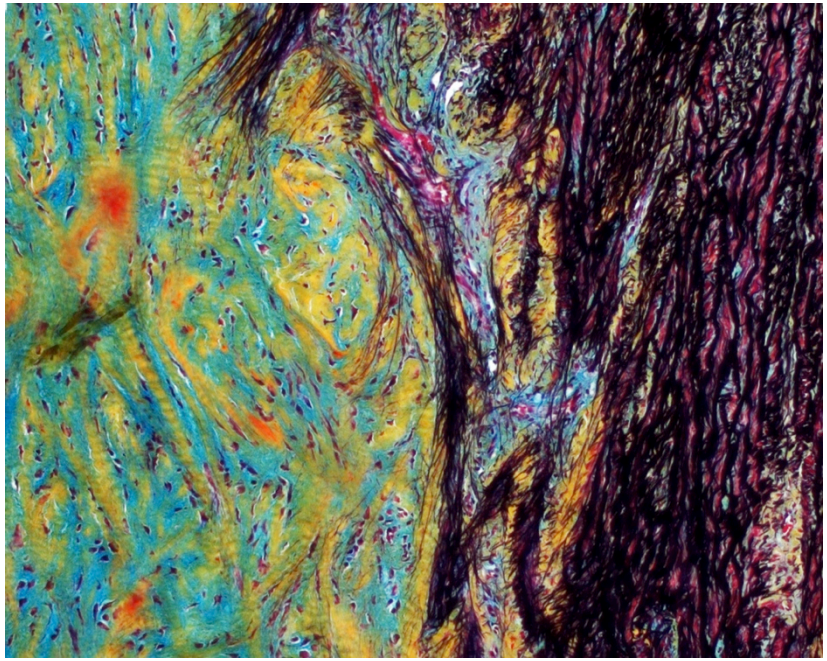
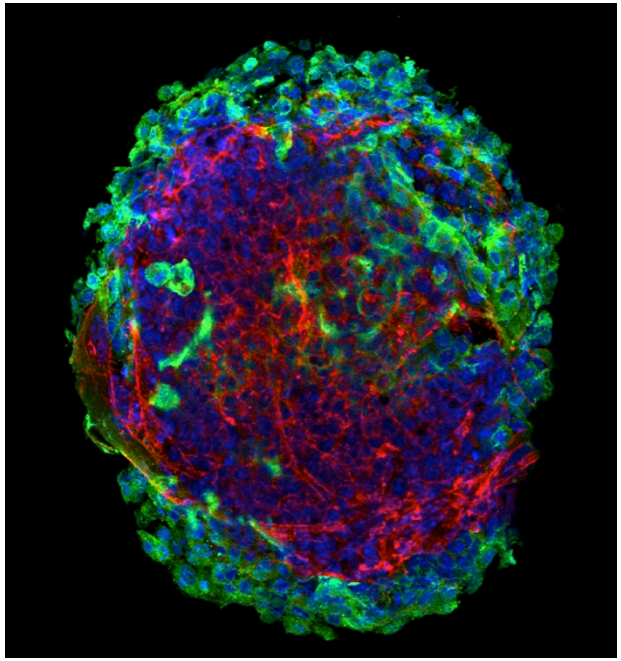
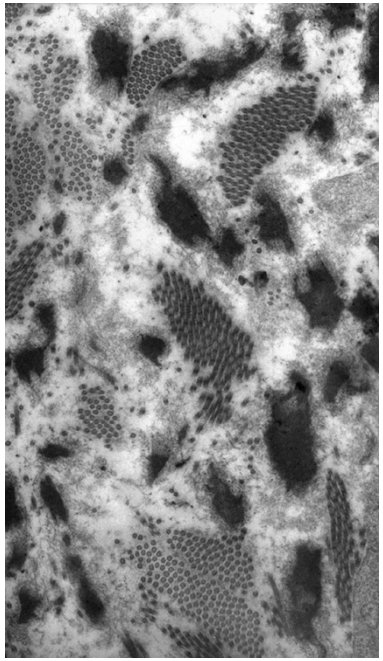
In vivo multiphoton imaging

Prof. Karsten König

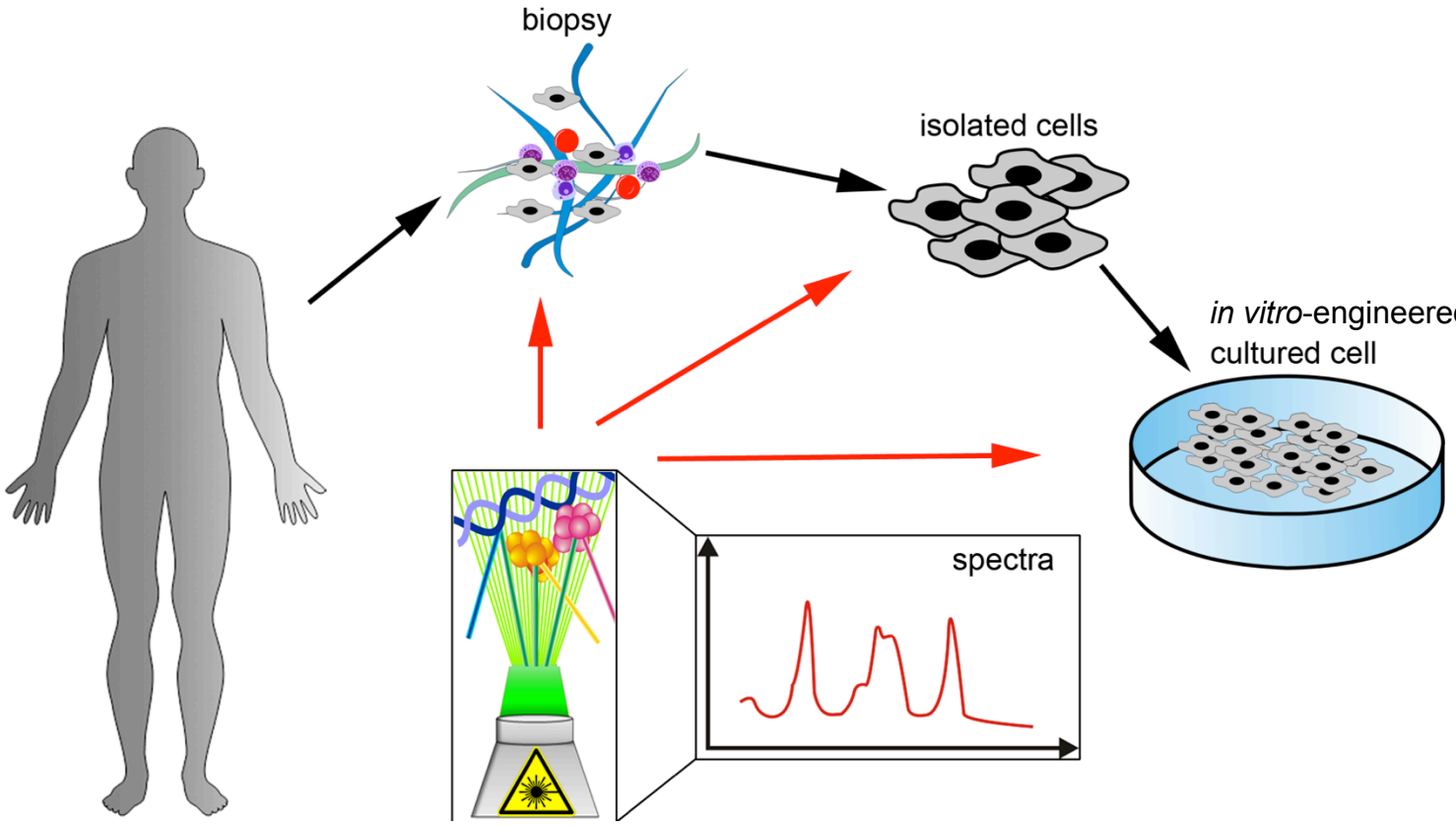


Non-invasive monitoring of cells & tissues

- Multiphoton imaging
- **Raman spectroscopy**

Raman spectroscopy



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Brauchle E and Schenke-Layland K
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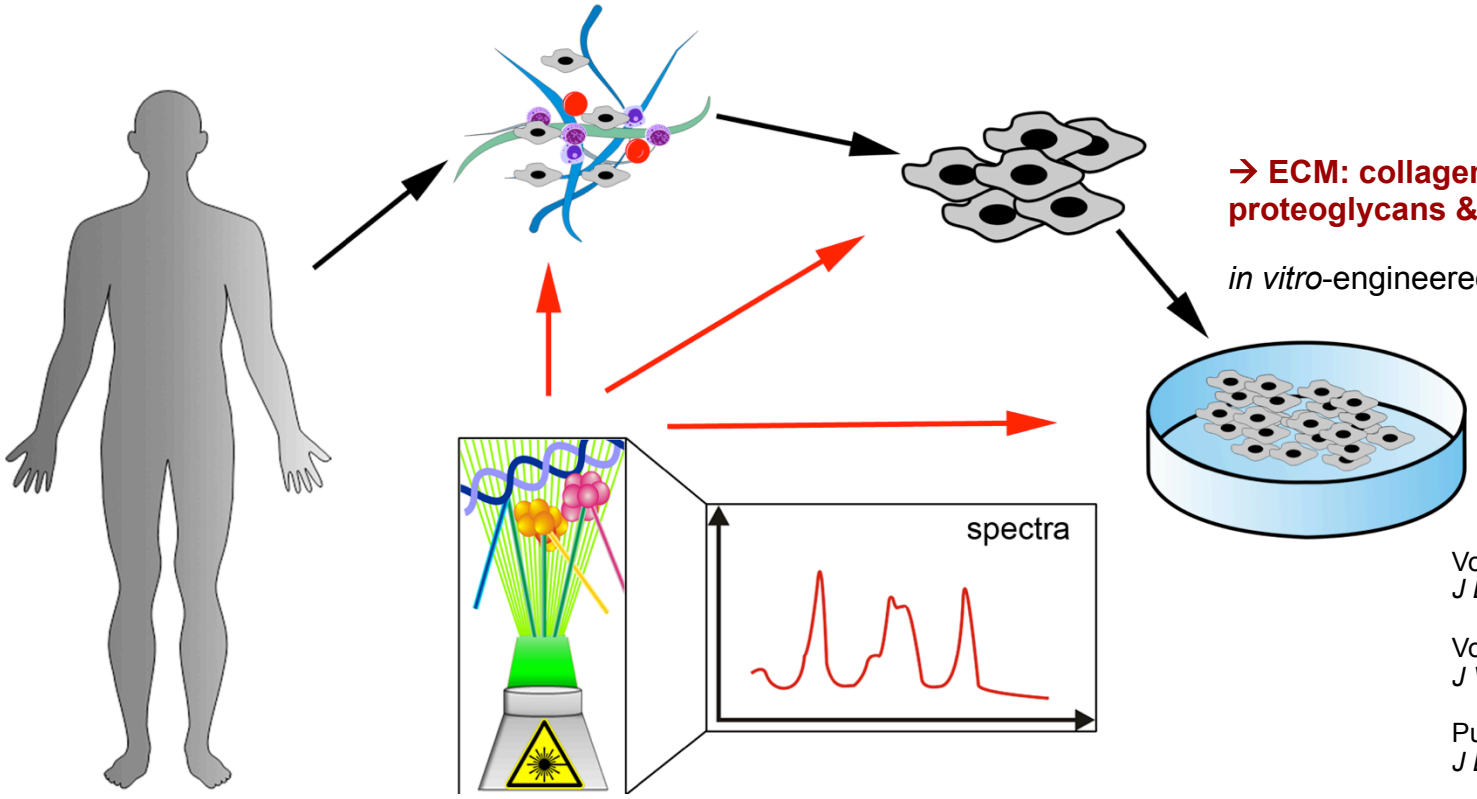
Raman spectroscopy



→ ECM: collagen & elastin fibers; proteoglycans & glycosaminoglycans

in vitro-engineered tissues & organs

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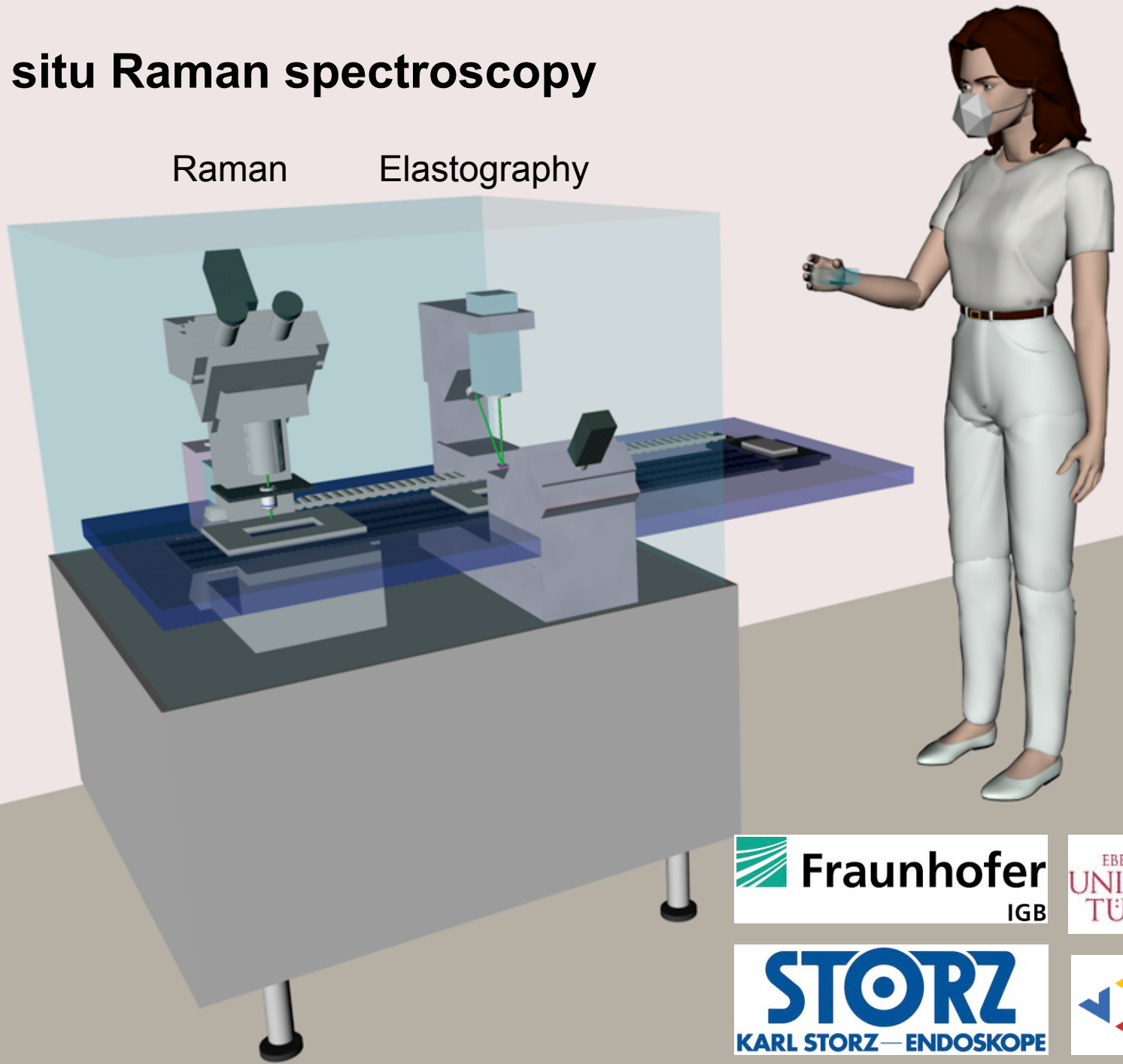
J Biophotonics 2012

Votteler M et al.
J Vis Exp 2012

Pudlas M and Brauchle E et al.
J Biophtonics 2012

Brauchle E and Schenke-Layland K
Biotechnol J 2013

In situ Raman spectroscopy



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Summary

- ImageStream^X mk II allows the combination of fluorescence microscopy and flow cytometry
- Cells and ECM can be monitored utilizing non-invasive and non-contact technologies such as multiphoton imaging (autofluorescence/ SHG/ FLIM) and Raman spectroscopy
- Raman spectroscopy can differentiate between cell phenotypes as well as primary-isolated and de-differentiated (pathological) cells, e.g. due to prolonged in vitro culture
- Raman spectroscopy is a sufficient method to monitor cardiovascular cell fate decision processes and for the non-contact, marker-free discrimination of cardiomyocytes

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Bundesministerium für Bildung und Forschung

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CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

SEVENTH FRAMEWORK PROGRAMME

MARIE CURIE ACTIONS

AMCARE
ADVANCED MATERIALS for CARDIAC REGENERATION

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Thank you!

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