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

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Tübingen, July 22\textsuperscript{nd} 2014
ImageStream$^X$ mk II
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A combination of Fluorescence Microscopy and Flow Cytometry

- Next generation instrument that overcomes limitations of both techniques

- ImageStream\textsuperscript{X} in Germany: 7 machines only so far no Core Facility

First ImageStream\textsuperscript{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™)
- 2 CCD Cameras
ImageStream\textsuperscript{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

- detect endogenous and exogenous fluorophores

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

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

Cells

760 nm Aortic Valve Leaflet

840 nm Aortic Valve Leaflet

König K. et al. Biomaterials 2005
Schenke-Layland K. J Biophotonics 2008
Brockbank KG et al. Cells Tissues Organs 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

- Biopsy
- Isolated cells
- In vitro-engineered tissues & organs
- Cultured cell
- Spectra

References:
- Pudlas M et al. *Medical Laser Applications* 2011
- Pudlas M et al. *Tissue Eng Part C* 2011
- Pudlas M and Brauchle E et al. *J Biophotonics* 2012
- Brauchle E and Schenke-Layland K *Biotechnol J* 2013
- Brauchle E et al. *Biomaterials* 2013

*In vitro*-engineered tissues & organs
Raman spectroscopy

$\rightarrow$ ECM: collagen & elastin fibers; proteoglycans & glycosaminoglycans

in vitro-engineered tissues & organs

Votteler M et al. J Biophotonics 2012


Pudlas M and Brauchle E et al. J Biophotonics 2012

Brauchle E and Schenke-Layland K Biotechnol J 2013
Summary

- ImageStream™ 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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Martina Seifert, Charité, Berlin
Heike Walles, University Hospital Würzburg, Germany

Thank you!

Anne Knopf  Dr. Ali Nsair

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