Correlative Microscopy

Applications and Solutions
Introduction to FEI

First sketch of a EM
Ernst Ruska (March 1931)

First TEM
Ernst Ruska & Max Knoll (1933)

Our company shipped its first TEM in 1949

Dr. Matthias Langhorst
60 years of Innovation

Transmission Electron Microscopes
TITAN

DualBeams™ HELIOS

Scanning Electron Microscopes
MAGELLAN

Dr. Matthias Langhorst
FEI worldwide

Semiconductors – Hillsboro, Oregon, USA

National Protein Science Facility – Shanghai, China

Dr. Daniel Shechtman wins the Nobel prize – Haifa, Israel

QEMSCAN WellSite Solution – Papua New Guinea
Overview
Founded 1993 by Prof. Rainer Uhl (LMU Munich)
Headquarters Graefelfing (Munich), Germany
Employees (2012) ~45 and growing

Since November 2011 a member of the FEI family!

Product Highlights
1993 Polychrome
1994 real-time imaging system FUCAL
2002 microscope platform iMIC
2004 TILL-TIRF
2011 spinning disk system Andromeda
2012 Intravital$^2$P
2012 CorrSight
The TILL portfolio
iMic systems

The iMIC:
• maximum light efficiency
• fully motorized + real-time control
• Truly equivalent ports for multimodal systems
• Flexible + modular concept

Unique Accessories:
• Polytrope: fast + flexible combination of modalities
• Yanus: best scanhead on the market
• Andromeda: Unique SD system
⇒ Powerful multi-modal imaging systems
The TILL portfolio
2P microscopy and Imaging systems

Specialized 2P system
- Highly efficient
- Perfect for intravital work
- Spectral detection system

Imaging systems
- Fast imaging systems build on TILL light source, real-time control and SW
- For variety of 3rd party stands
Fluorescence microscopy allows
- Specific labelling with high sensitivity
- Imaging of living specimens
But: Do you know precisely where the fluorescence label resides?

Electron microscopy offers
- Open view of structural stainings
- Ultra-high resolution of electron microscopy
But: The sample is dead and specific staining is complicated

Now imagine you could have both...
CLEM

The benefits of challenging experiments

Add Orientation
- Put high resolution EM data into low magnification context

Save Time
- Pre-screening at low magnification
- Use of additional contrasts to identify rare events
  - Fluorescence, phase contrasts, ...

Add Information
- Correlate time-lapse imaging of dynamic event
- Correlate functional LM imaging (pH, ion-concentrations)
  with ultrastructural information
Add Orientation
Maintain context throughout the workflow

Light microscopy offers:
- Imaging at different magnification scales
- Easy adaption to different sample geometries

⇒ Document the origin of your EM sample throughout the workflow
⇒ Put the high resolution information of the EM into context

Light microscopy offers:
- Low magnification overview
- Specific staining of target cells / structures using different labelling approaches
- Easy screening of several samples

⇒ Select promising samples
⇒ Identify regions of interest
⇒ Save coordinates and transfer to EM
⇒ Start EM imaging „knowing where to look“

⇒ Increases specificity and sensitivity of EM imaging!

Light microscopy offers:
- Dynamic imaging of cells and tissues in the native state
- Specific genetic labeling
- Functional labels for read-out of biochemical parameters (e.g. Ca$^{2+}$)

⇒ Collect time lapse information
⇒ Process your sample for electron microscopy
⇒ Relocate to the area of interest
⇒ Collect high resolution information of the same cells
Challenges in CLEM

CLEM experiments are often cumbersome and time-consuming

You have to:

- Choose and optimize the workflow
- Gather enough data for statistical significance
- Develop expertise on sample preparation, light and electron microscopy

Live sample

Fixation

Processing

EM imaging

Intact + living

Sliced + vacuum-compatible
The biggest challenge in CLEM

Adapted from slide of Bruno Humbel, UNIL Lausanne
iCorr™: Full integration of a light microscopy module without compromised on TEM performance

Widefield light microscope:
Objective 15x/0.5
Reflectance and Fluorescence imaging

Quick and easy switching between FM and EM mode:
- FM imaging at 90° stage tilt
- EM imaging at 0°

Instant localization of ROI in EM
Fluorescence signal vs. EM contrast

Imaging fluorescence and ultrastructure on two different instruments along the workflow allows:
- Optimization of labelling + best performance in both modalities
- Live cell imaging on the light microscope
- Screening of a large number of samples
To correlate data sets from two different instrument, a common coordinate system for precise overlay has to be established!

⇒ Markers visible in both modalities are needed!

- Fiducials on sample carrier
- Markers written into the sample structure
- Correlation on features in the sample
  - Beads, nuclei
Relocation in a non-integrated CLEM experiment

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• Precision of overlay depends on distance between marker and structure of interest!
  ⇒ rough alignment by sample carrier fiducials, fine alignment on structures close to ROI

• Sample preparation can induce changes (e.g. shrinking)
  ⇒ corrections needed, but care has to be taken not to “overcorrect”
CorrSight live opens up a simple workflow from live cell imaging to 3D electron microscopy of subcellular features.
Relocation through Processing Steps

Rough landmarks like grids, fluorescent beads etc. can easily be lost during processing of the sample (e.g. sectioning)

⇒ Unless these landmarks are used to guide the processing steps!
  ⇒ Trim to size according to landmarks
  ⇒ But leave enough of “old features” visible to re-establish the coordinate system
MAPS
An open SW interface for CLEM
MAPS: Image-based correlation

- Correlate purely on image based information
  - Complete freedom, use fiducials, markings or sample features
  - No hardware dependencies
- 1, 2 or 3 point alignment possible
- Automation possible whenever location of fiducials is known
- Multiple fine alignments possible for high resolution correlation
- Data is organized in layers and image pyramids for easy access
CorrSight™
A dedicated light microscope for CLEM
CorrSight™ will greatly enhance your productivity in CLEM experiments!

- Only purpose-built instrument for CLEM with clear interfaces for easy adaption to different workflows
- **Highest data quality** by high degree of automation and dedicated solutions like integrated microfluidics for reproducible sample processing
- Only CLEM solution with control of both light and electron microscope by one single, easy-to-use software package
FEI’s Vision on Cell Biology Workflows

Collaborations

Sample Preparation  LM Data acquisition  EM Data acquisition  Data Correlation  Information extraction

Living Specimen  Data Processing  Fixed Specimen
Thank You

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