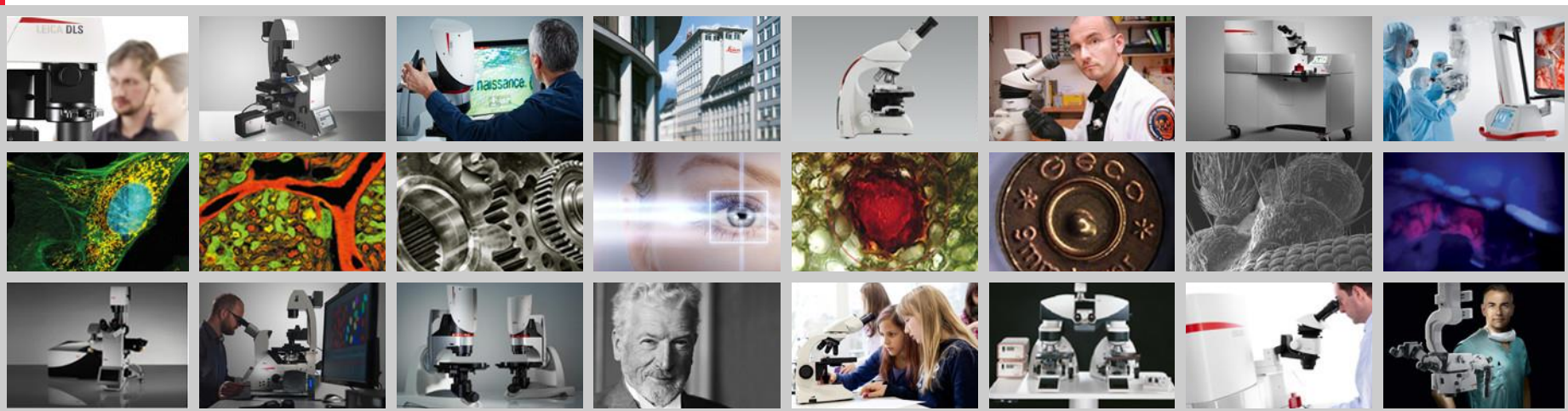
From Eye to Insight



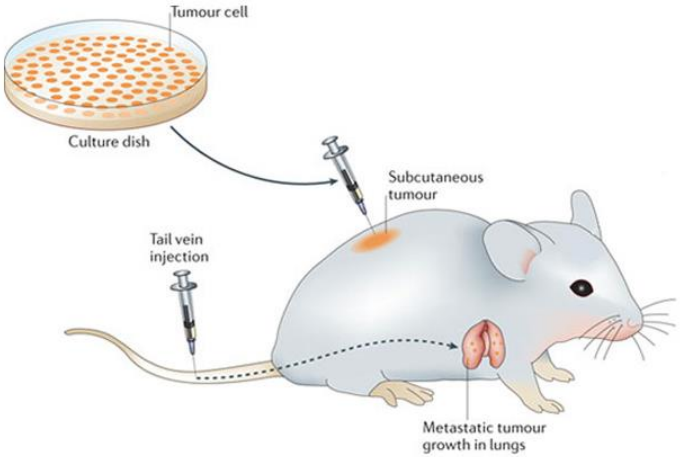
# Workflow – Spheroids: 3D tissue model in cancer research

Irmtraud Steinmetz

Science Lab

Updated by: Marco Meijering
Application Support Specialist EMEA

# 2D cell culture and animal models for Cancer research




Adapted from Dranoff Nature Reviews Immunology, 2012

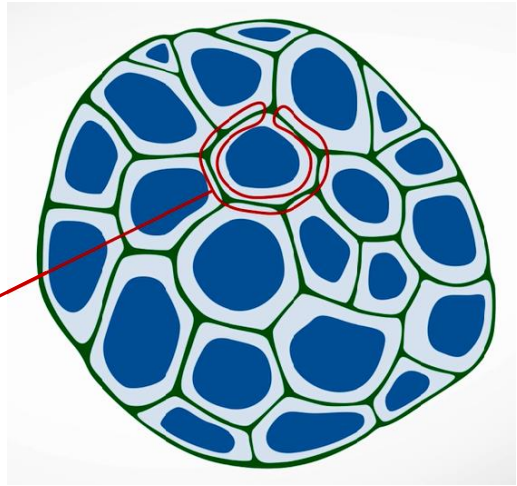
**2D- cell culture**

- Lack of realistic 3D- complexity
- high drug failure rate

**animal models**

- expensive
- time consuming
- frequently fail to reflect human tumor biology

# Alternative: 3D cell culture/spheroids

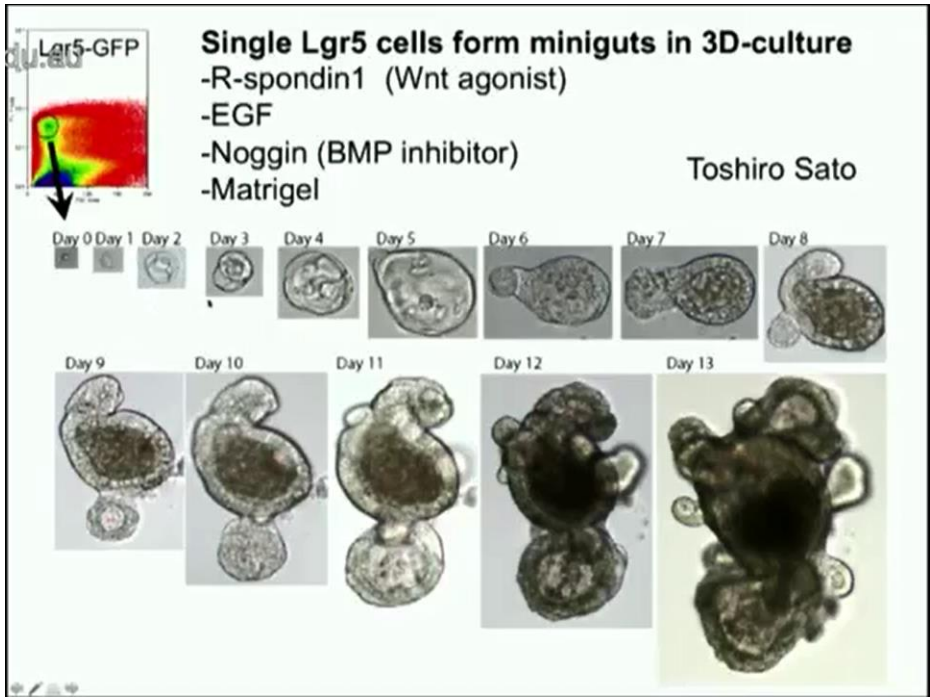



- Characterized by contacts to other cells in xyz, cell signalling, cell-polarization, interaction with extra cellular matrix (ECM)
- 3-D-architecture develop physiological metabolic and proliferative gradients
- good compromise of expensive animal models and non physiological 2D-cell culture
- can serve as physiological models for cancer and stem cell research

# 3D Biology in stem cell and cancer research and other diseases – exploding field

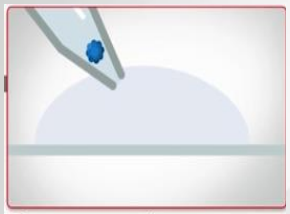
**Research with spheroids**

- Drug screening, drug sensitivity resistance behaviour of cancer cells, tumour genetics
- Personalized Medicine: so far for colon, breast and pancreas cancer
- Cure of cancer, cystic fibrosis, …
- Idea: built up a living cancer bio-bank (Hans Clevers, Utrecht, Netherlands)
- Organ Modelling




Talk:
Hans Clevers (2015): Wnt signalling, Lgr5 stem cells, organoids and cancer at
Walter and Eliza Hall Institute of Medical Research, Australia

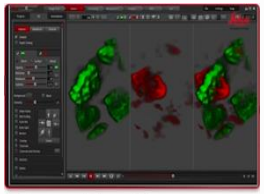
# Workflow - Most efficient Imaging of 3D Spheroids



Preparation

Acquisition

Archiving

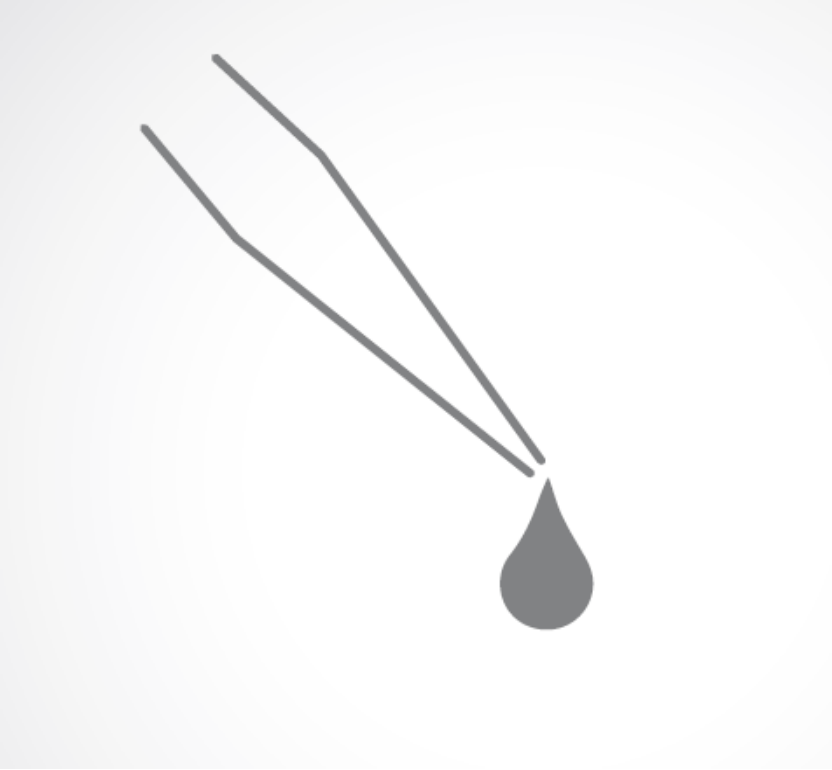


Visualization

Analysis

Share

# Preparation



# Preparation – Starting with 2D cell culture

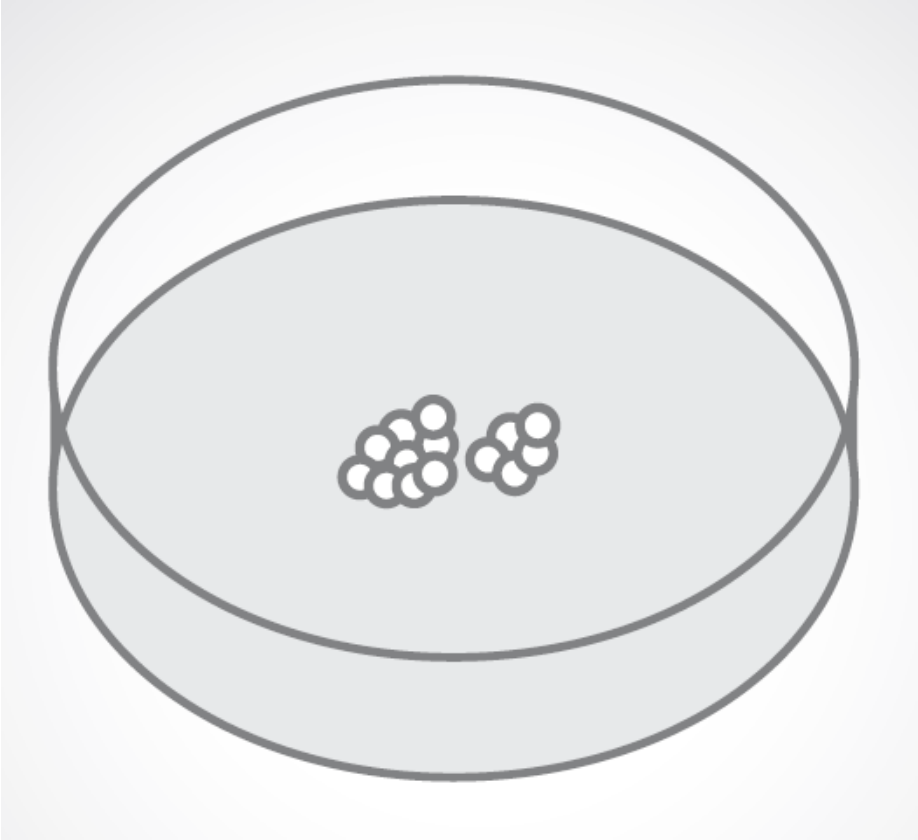
- 2 D Cell Culture
- When the 2D-cell culture is ready for preparing the spheroid growing
  → Classical 2D-cell splitting protocol with trypsin
- Cells in PBS (buffer solution)

**Standard breast cancer cell lines**

**e.g. Cell Type Example: MCF10A-cells**

commercially available cell line, human breast epithelial cells, immortalized, non-tumorigenic, cell cycle time 20-21hours.

# Mounting



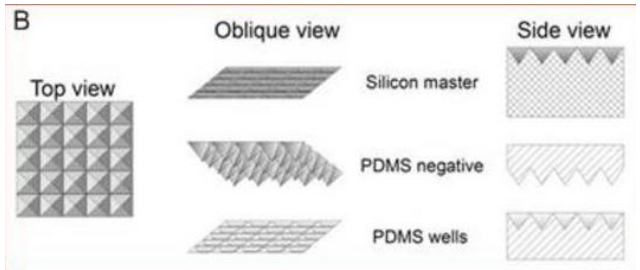
# Mounting – How to grow the spheroids?

**Gels**

soft tissue-like stiffness of gel that mimics Extra Cellular Matrix (ECM)

Refractive index close to water:

- **Matrigel**
- Hydrogel
- Agarose (low conc > 1%)
- Phytagel, for building holders

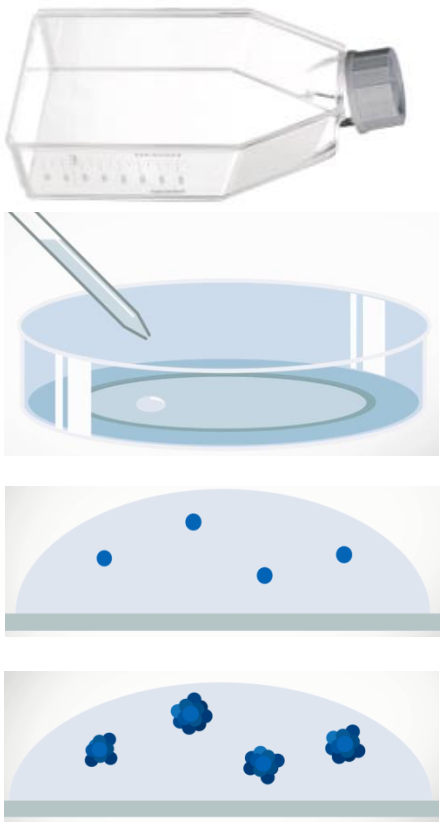



Moulds to get spheroids of the same size

**Scaffolds**

- 3D matrices made from different materials reflecting the *in vivo* ECM of particular tissue

# Mounting – Growing spheroids



„hanging drops“

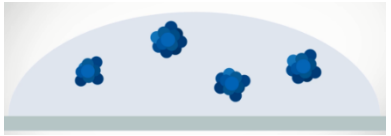
**Short Protocol: Cells Prepared for mounting and subsequent cultivation for development of spheroids**

- Cells in solution
- Mix cell solution with Matrigel to get a 90% Matrigel concentration:
- 6-7µl of cell solution add 70 µl Matrigel (Matrigel needs to stay on ice)
- Put mix in Eppendorf tubes, mix , put on ice again
- Suck cells in 1.5 – 2µl drops
- Place several drops on the glass bottom of the petri dish (3.6 mm diameter) in a row
- Turn petri dish upside down to let the spheroids sink into a position of optimal observability, cells are kept in position
- Place in an 37°C incubator for 10min to polymerize the Matrigel.
- Add culture medium and place dish in the incubator for development of spheroids

- Single cells need to be mixed with a drop of gel (Matrigel)
- Each cell can grow to a spheroid, several spheroids in one drop

# Mounting for Imaging with the DLS

1



2

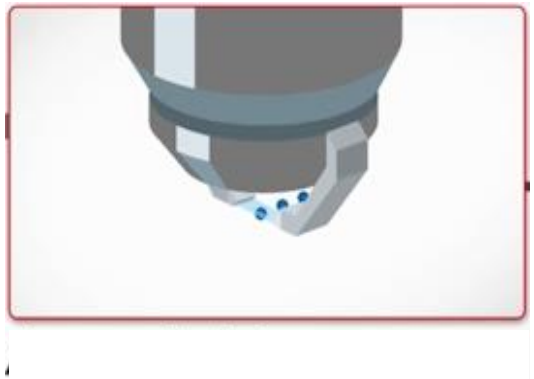
- **Advantage:** Imaging directly several spheroids within one drop

or

- Transfer developed spheroids in a drop of gel (hand-picking)
  several drops, perfect positioning!

- **Advantage:** Several drop possible in a row

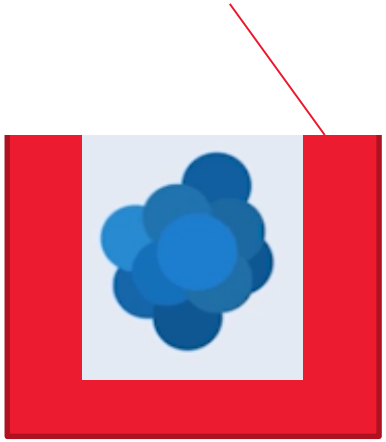
- Simple preparation!



# Option 2: Cell clusters are established elsewhere

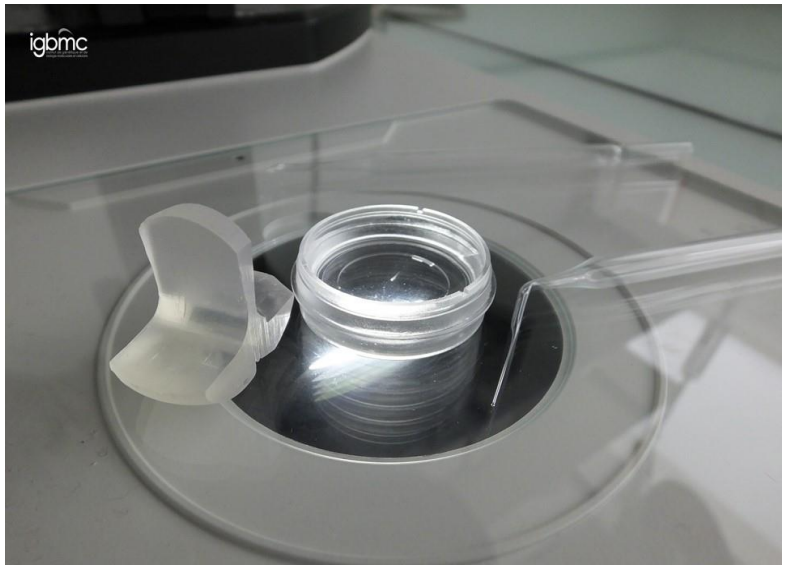
Are imaged in a „groove" filled with Hydrogel / Matrigel

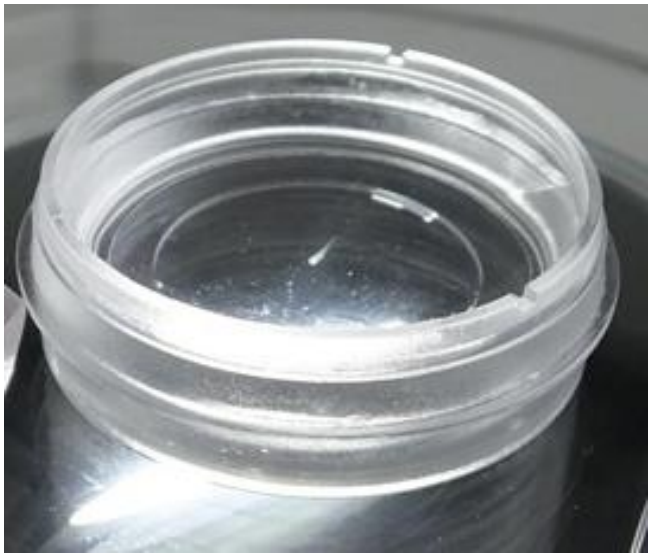
Glass / Agarose



# Option 3: Cell clusters are established elsewhere

Are imaged held by walls of Phytagel shaped by mould



Developed @IGMBC

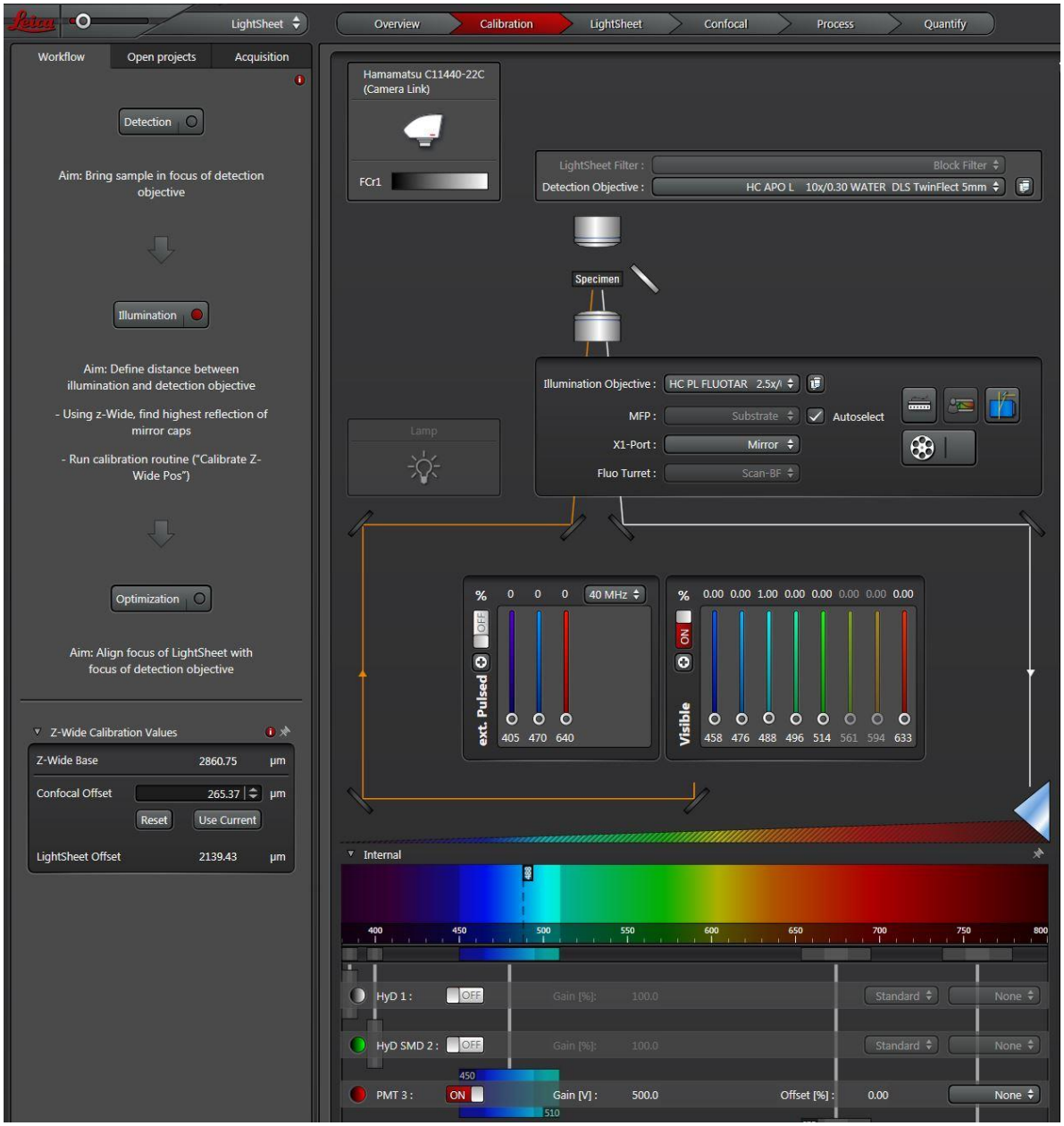
# Acquisition

# Acquisition – LAS X for DLS

**DLS**

**Advantages:**

- Calibration routine as workflow
- Intuitive User Interface



# Acquisition – The best Method

**Light Sheet Microscopy**

- High speed (sCMOS – camera)
- Low light exposure
- Most physiological way of observation

  helps to prolong cell viability and prevents the sample from photo toxic effects
- Several spheroids can easily be imaged one after the other

Optics: 25x0.95 detection lens, 5mm TwinFlect mirror device, 2.5x illumination lens

| | | | | | |
|---|---|---|---|---|---|
| Illumination (w BE) 2.5x/0.07 | | | | | |
| Detection 25x/0.95 | Thickness: 3.6µm<br>Length:~240µm | 330 nm | <1µm | 2.5mm | 295µm x 295µm |

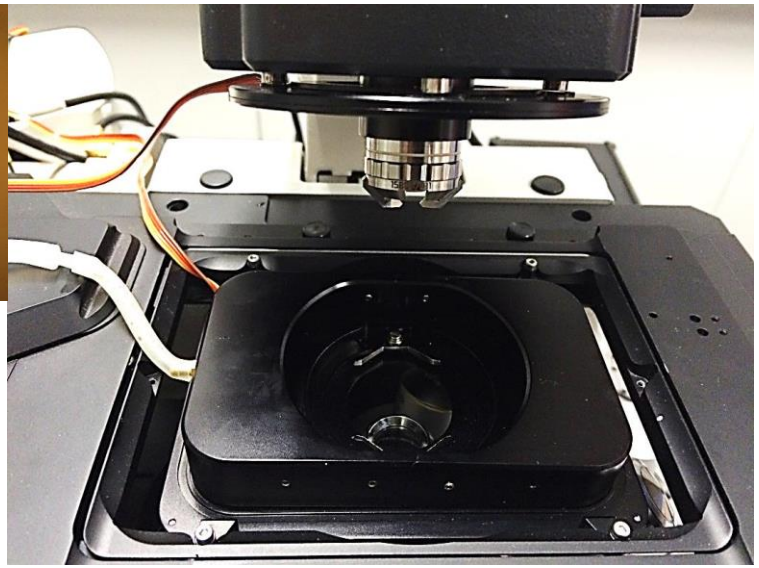
# Acquisition – Climate Control

Requirement for observation of live spheroids: Constant 37° C and 5% $CO_2$, humidity 100%

Options on SP8-DLS-system:

- Ludin-Box (LIS)
- TokaiHit top stage incubator
- OkoLab stage incubator **NEW!**

Settings for climate control in LAS X



# Acquisition - Protocol

**Speed, Format etc.**

- Select xymzt scanning mode
- Camera – exposure time
- Lowest laser power possible
- Fast switching between excitation wavelength due to quadruple filter
- Draw ROI (e.g. 100x100µm) – increases speed!
- Define 100 µm Z-stack to image through whole spheroid, 0.37mm Z-step size
- In LS: move Z to top and mid

**Multiposition Settings**

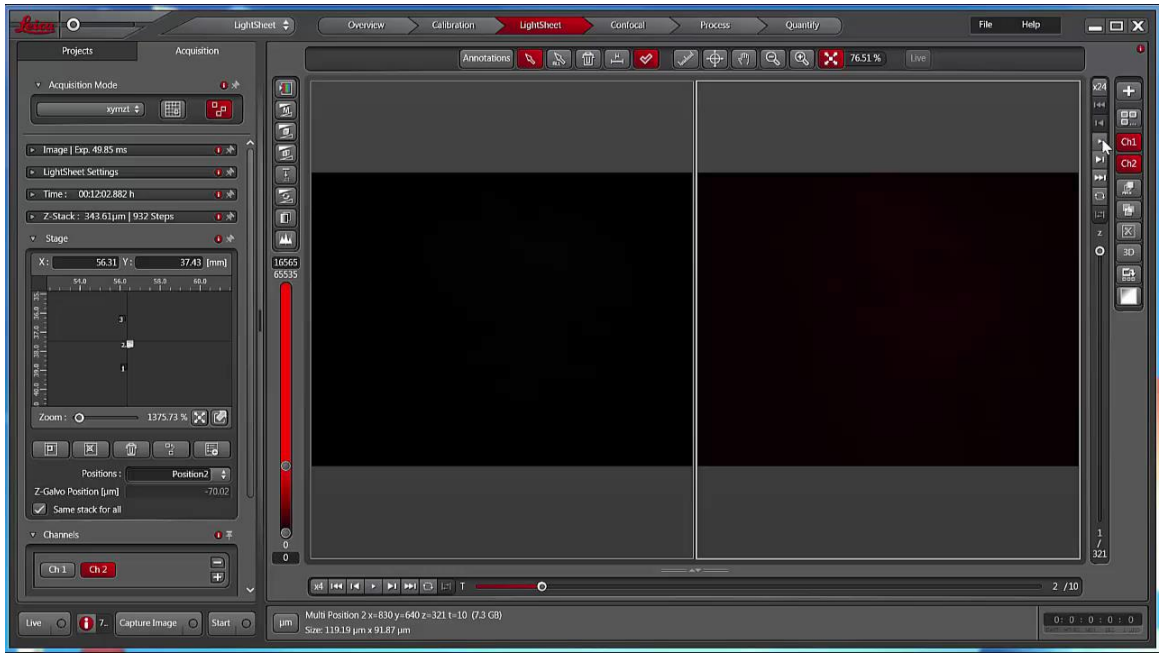
- Open Mark and Find window, mark position of actual spheroid in focus- in scattering mode (less light necessary!)
- Put next spheroid in focus, re-center, mark, etc for several positions
- Acquisition: merge offline
- Imaging at least for 4h – 5h to see first mitosis
- Also 1 mirror for acquisition is OK, for 2 mirrors better online merging

# Acquisition – Imaging Settings

**Settings for the fluorescent labels:**

**1. Setting: 488nm** for endogenous H2B-GFP (green channel); labels histone subunit for studying cell division in the living spheroid

**2. Setting: 638nm** for SiR tubulin (red channel); fluorescent marker in the far red range, (Spirochom dyes) for tubulin, mitotic spindles




**Courtesy:**
Christian Conrad and
Björn Eismann
BioQuant, Heidelberg

# Archiving

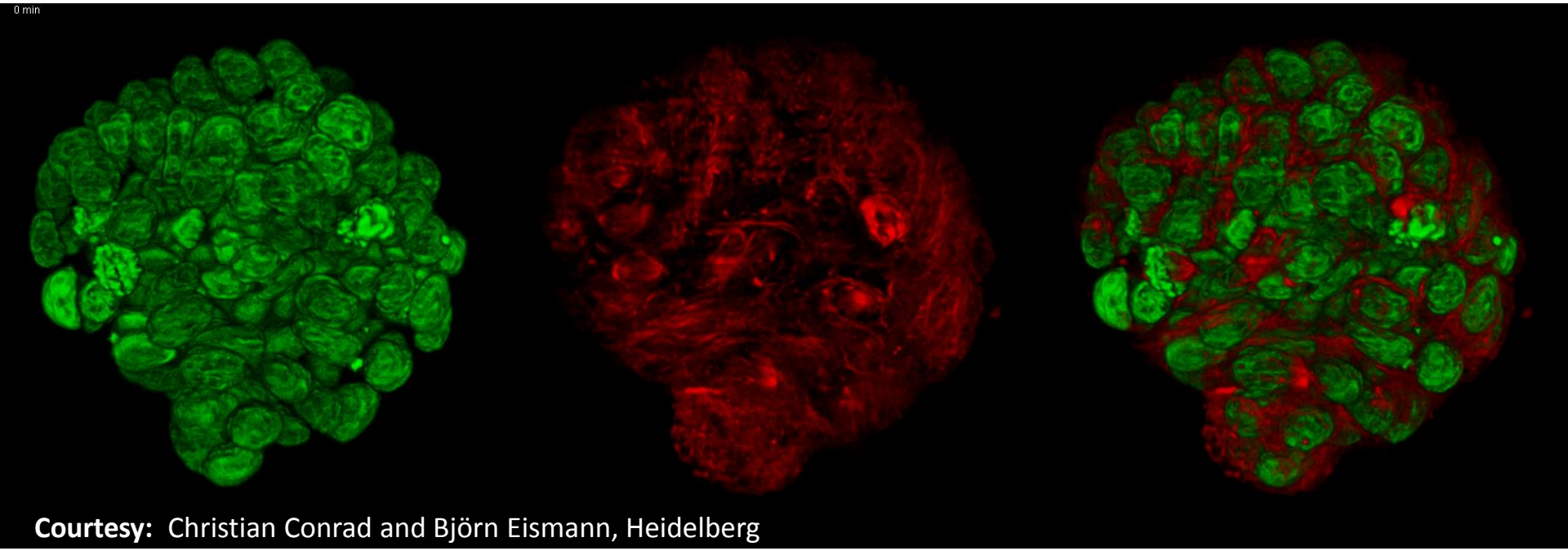
# Archiving

- Autosave – user defined the location for data saving
- Fast data streaming from temp memory to a hard disc / data server
- Save only merged data (from left and right mirror)

# Visualization

# Visualization – The LAS X 3D-Tool

**Green:** endogenous H2B-GFP **Red:** tubulin SiR tubulin

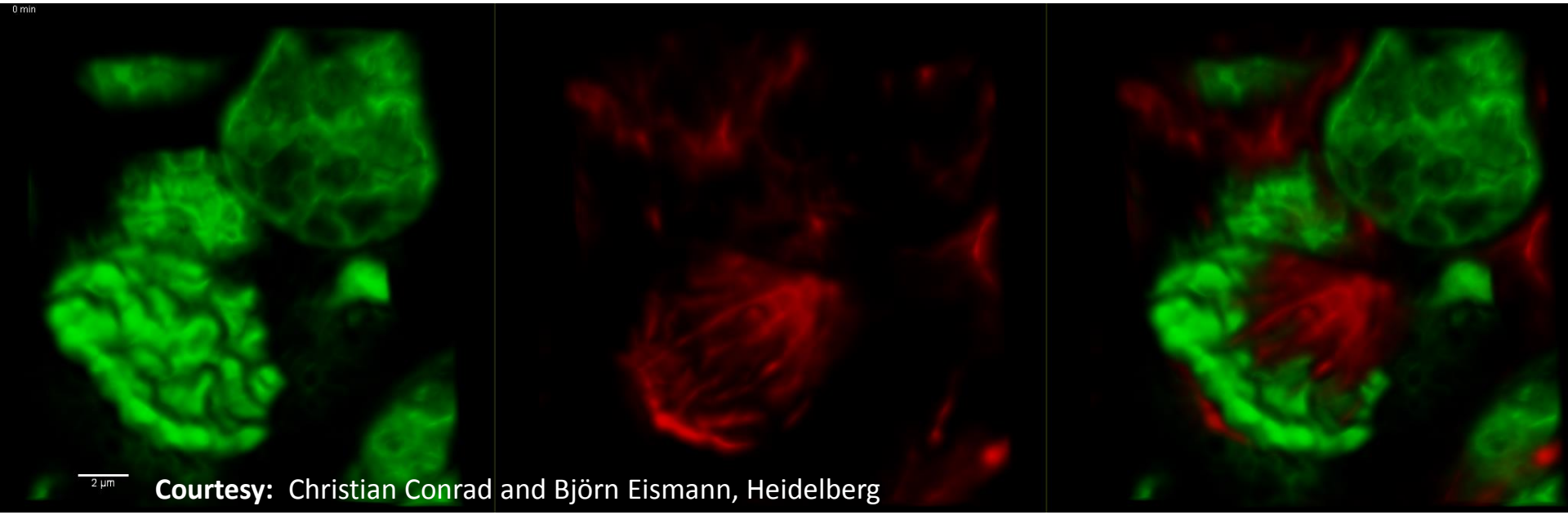



**Courtesy:** Christian Conrad and Björn Eismann, Heidelberg

- Direct visualization of data with the LAS X 3D-Tool – one click!
- Pyramid format for fast navigation within the 3D-tool – optimized for large data sets
- Rendering 3D over time with 2 mirrors (xymzt)
- Movie Creator, Clipping, Sections, Cropping, …….

# Visualization – Concentrate on Details

**Green:** endogenous H2B-GFP **Red:** tubulin SiR tubulin

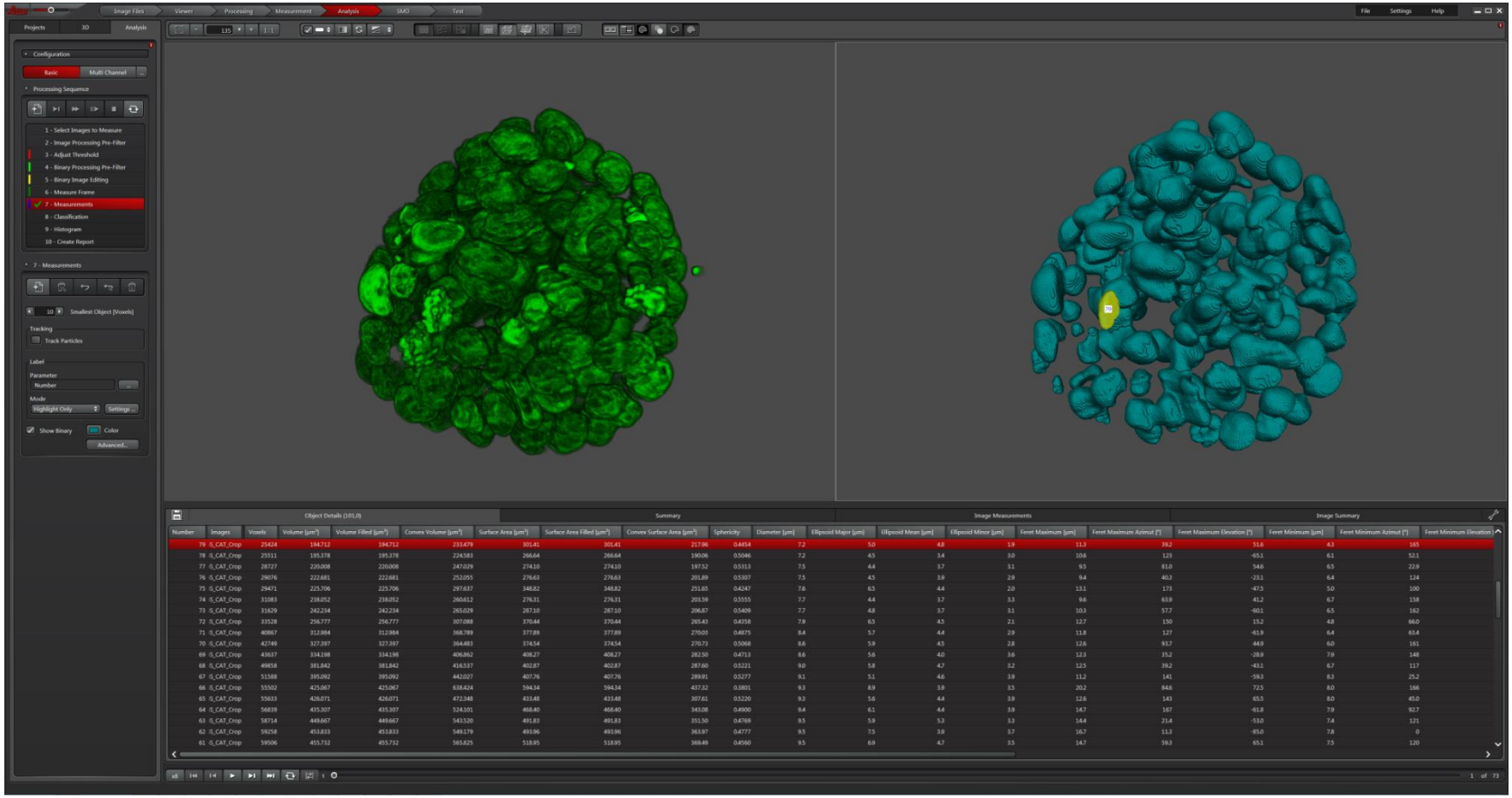



**Courtesy:** Christian Conrad and Björn Eismann, Heidelberg

- Crop out sub volumes to catch up up cell divisions
- SVI Huygens for deconvolution of light sheet data

# Analysis

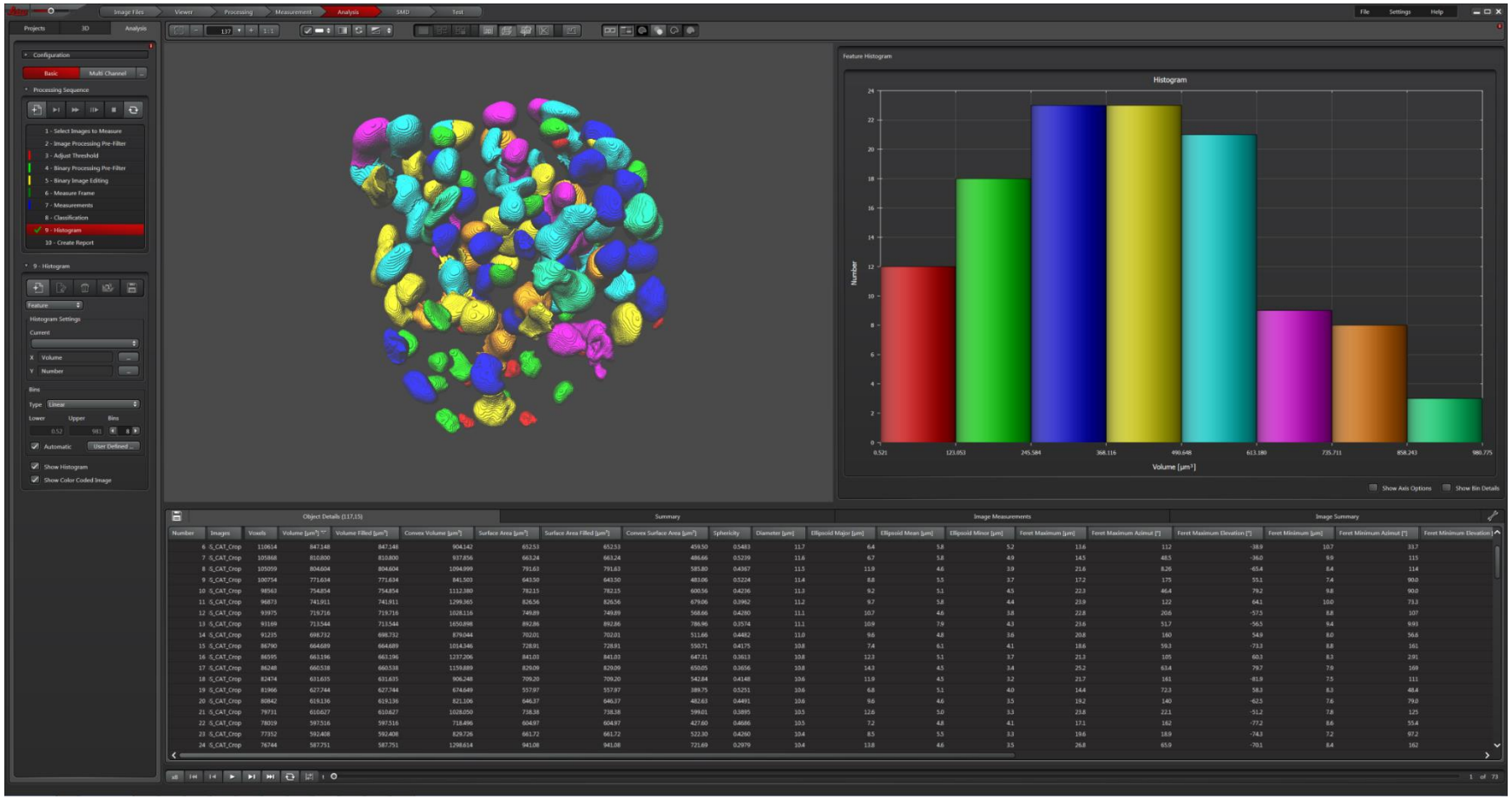
# Analysis – User defined Processing Sequence




- User defined Processing Sequence – its also a workflow:

Pre-Filter, Thresholding, Binary-Filter, Binary Image editing, Measure Frame, Measurements, define Classification (numbers, volumes, areas, diameters, form factors etc.)

# Analysis – Results ready to use

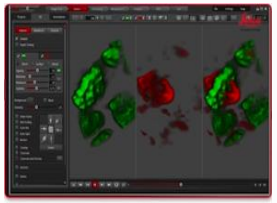



- Get histogram, Create report
- Share all data and results

Preparation

Acquisition

Archiving



Visualization

Analysis

Share

Leica
MICROSYSTEMS

# From Eye to Insight