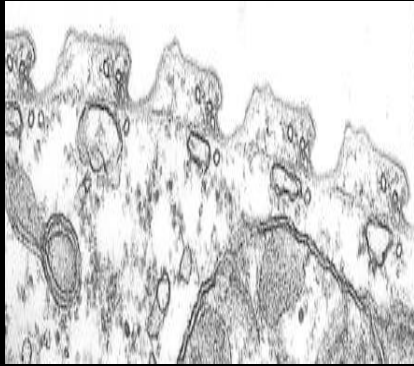


Features and Architecture

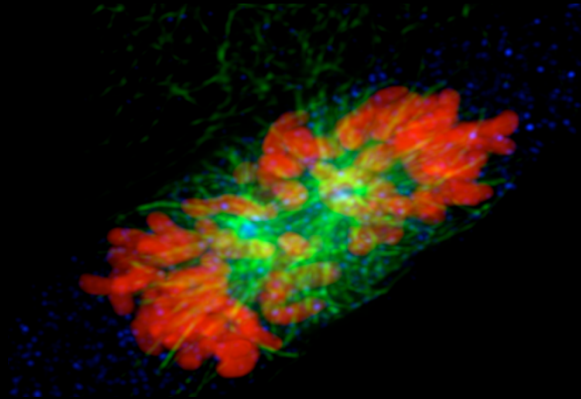
**OMERO**

# Image Problem

A pretty picture, or a measurement?



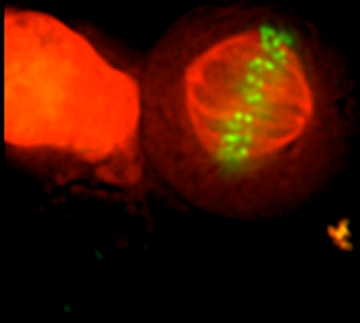
Organelles



Cells



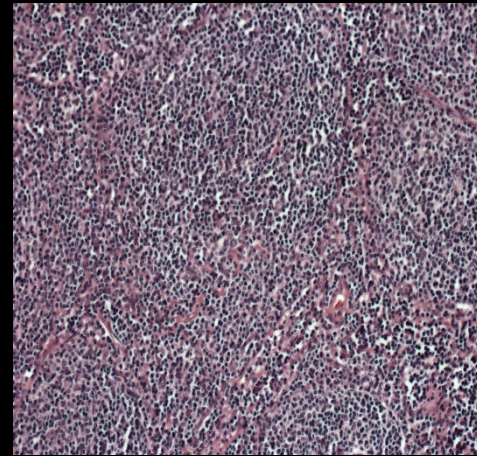
Retinal Imaging



Dynamics



Physiology



Pathology

# Imaging Modalities

- Fundus Camera
- Optical coherence tomography
- Fluorescence
- Histology
- High Content Screening
- Fluorescence Lifetime Imaging
- Atomic Force Microscopy
- Electron Microscopy
- Dicom – from Bio-magnetic Imaging to Ultrasound

# Imaging Modalities

- Overview of types of imaging
  - Objective
- Data
  - Type
  - Dimensionality
  - Size
- Analysis
  - Complexity
  - Methods

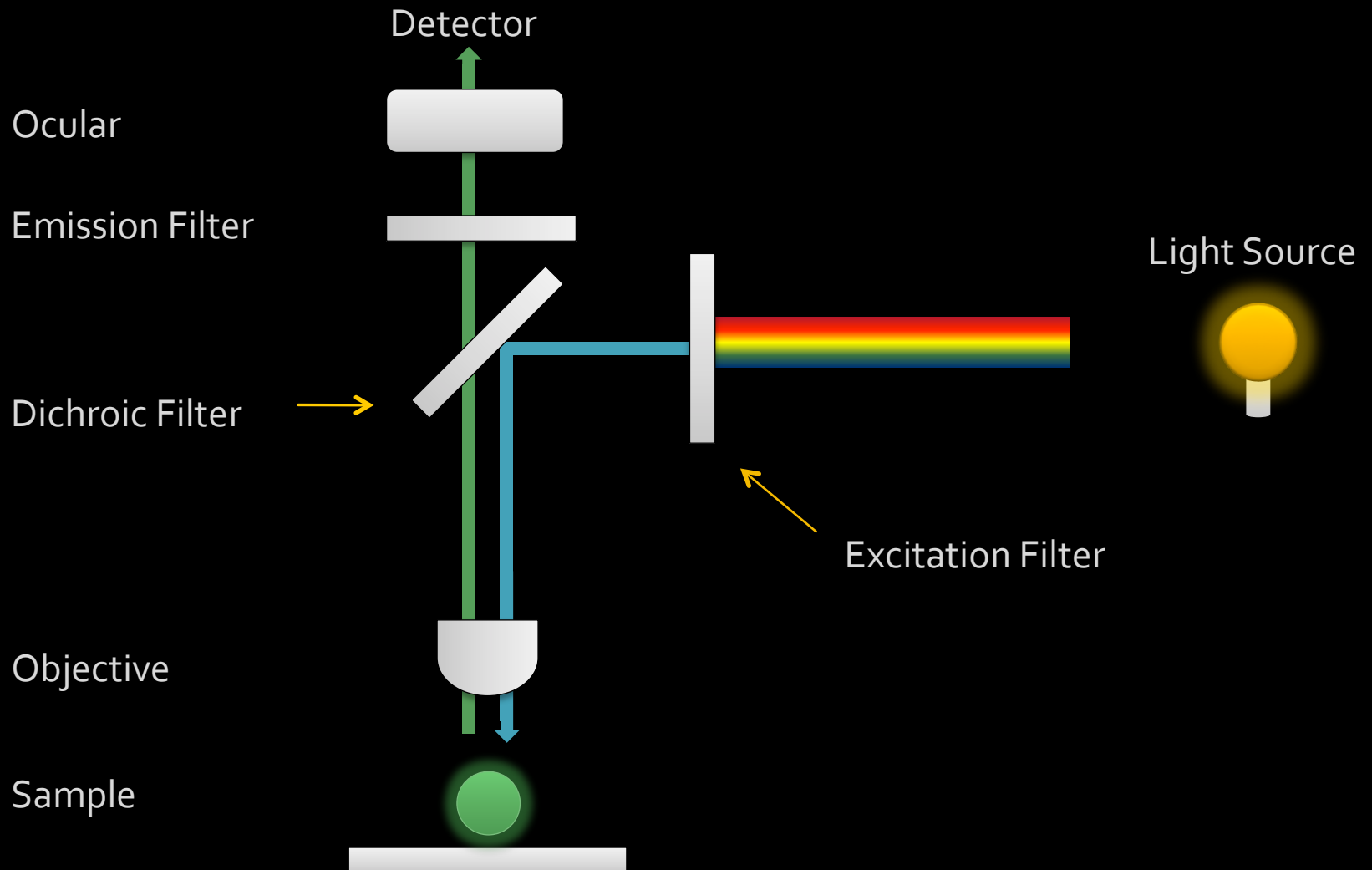


# Fluorescence Microscopy

## ■ Overview

- The specimen is illuminated with light of a specific wavelength (or wavelengths) which is absorbed by the fluorophores, causing them to emit light of longer wavelengths (i.e., of a different colour than the absorbed light).
- The illumination light is separated from the much weaker emitted fluorescence through the use of a spectral emission filter. This emitted light is stored as channels.
- Multi-colour images of several types of fluorophores must be composed by combining several single-colour images.

# Fluorescence Microscopy



# 5D Images

- X,Y
  - typically 512, 512 or 1024, 1024 but now seeing larger.
  - 30K, 30K common in pathology images.
- Z component
  - microscopes have a depth of focus meaning they can see into a sample by a few microns.
- Time component
  - Time lapse images are common in cell biology and Fluorescein angiography.
  - Timescale: up to 72 hours.
- Channel component
  - In fluorescence microscopy proteins can be tagged with a dye that fluoresces at a particular wavelength.
  - In AFM microscopy there can be many non-image features recorded.
  - It is typical to have 3-4 channels in an image, though some imaging techniques can have 30+.
- Bit depth
  - Typically 12 bit, but can be 8, 16, 32, float, double or complex

# Fluorescence Microscopy - Data

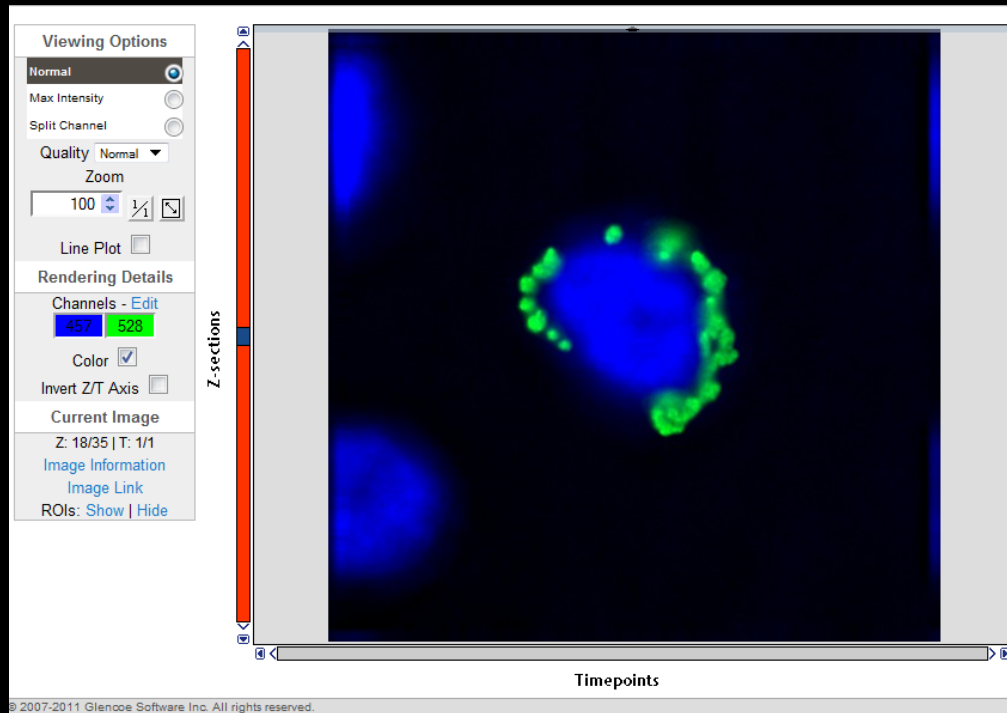
- Type: 5D Images
- Data type: typically 8, 12 or 16bit
- X, Y: 512, 512 now moving on to 2048, 2048
- Z: In fixed cell can be 64+, less in live cell imaging
- T: 0 in fixed cells, can be 1000+ in live cell imaging
- C: 3-4 typical, can be more.
- Size: 8MB-20GB

# Fluorescence Live Cell Imaging

- Objective

- An increasing number of investigations are using live-cell imaging techniques to provide critical insight into the fundamental nature of cellular and tissue function, especially due to the rapid advances that are currently being witnessed in fluorescent protein and synthetic fluorophore technology.
  - cell biology
  - developmental biology
  - cancer biology
  - many other related biomedical research laboratories

# Fluorescence Microscopy - Image



# Fluorescence Microscopy – Analysis

- Super Resolution Methods
  - PALM/STORM
- Analysis
  - Deconvolution
  - Life cycle detection
  - Cell death count
  - Particle tracking
  - Similar phenotypes

# Fluorescence Lifetime Imaging

- The fluorescence lifetime is the signature of a fluorescent material
- The exponential decay in emission after the excitation of a fluorescent material has been stopped.
- FLIM (Fluorescence Lifetime Imaging Microscopy) is a technique to map the spatial distribution of lifetimes within microscopic images and it allows measurements in living cells as well as in fixed materials.



# FLIM - Objectives

- Some phenomena do affect fluorescence lifetimes, the lifetime is used to detect these phenomena leading to various applications including:
  - ion imaging (pH measurements)
  - oxygen imaging
  - probing microenvironment
  - medical diagnosis.
  - Co-localisation
  - One of the most powerful FLIM-application in biology is Fluorescence Resonance Energy Transfer (FRET).

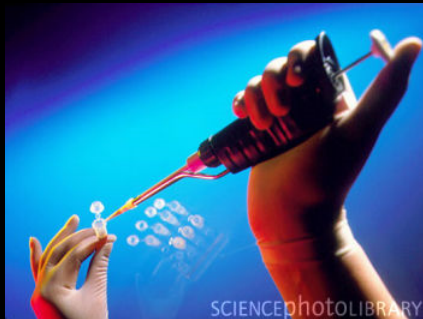
# FRET - Fluorescence Resonance Energy Transfer

- When two fluorescent molecules (or two fluorescent labeled epitopes within a protein) are in very close proximity, i.e. less than 9 nm, the energy of the one fluorescent (donor) molecule (e.g. GFP) is transferred in a nonradiative process to the other fluorescent (acceptor) molecule (e.g. mCherry). In this way, the lifetime of the donor molecule decreases and this change can be measured quantitatively by FLIM.
- Interaction

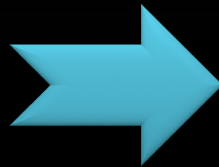
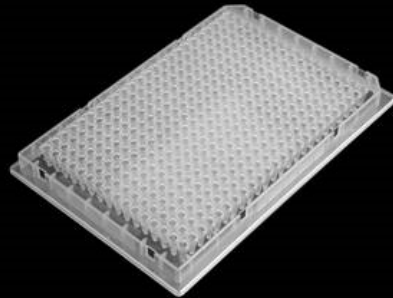
# FLIM - Data

- Type: N-D Images
- Data type: typically 8, 12 or 16bit
- X, Y: 256, 256 now moving on to 1024, 1024
- Each pixel has a time series; decay histogram.
- T: Can have multiple time points
- C: 1 typical, can be more.
- Size: 32MB+

# High Content Screening

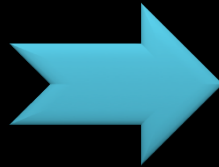


Pipettes and vials



96/384 well plates and robot control

# High Content Screening



# High Content Screening

Cells



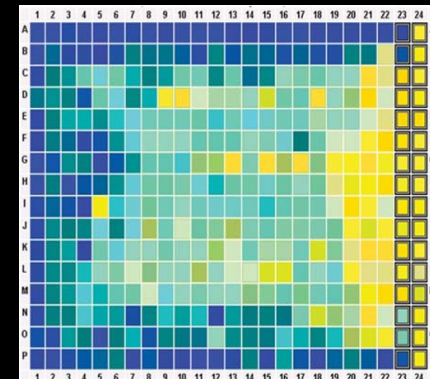
Image Data



Numerical Data

| Data Table |                |                 |                   |      |
|------------|----------------|-----------------|-------------------|------|
| File       | Mean_Nuclei_Ar | Mean_Nuclei_Int | ImageNumber       | well |
| 134.614    | 47.18          | 47.18           | 4 NM_008067-...   | 11   |
| 188        | 47.61          | 47.61           | 42 NM_176942-...  | 11   |
| 191.328    | 45.871         | 45.871          | 43 NM_176942-...  | 11   |
| 194.396    | 47.629         | 47.629          | 44 NM_176942-...  | 11   |
| 173.482    | 45.416         | 45.416          | 57 NM_176942-...  | 15   |
| 153.477    | 40.918         | 40.918          | 60 NM_176942-...  | 15   |
| 137.067    | 40.693         | 40.693          | 82 NM_008066-...  | 21   |
| 136.368    | 41.549         | 41.549          | 84 NM_008066-...  | 21   |
| 134.496    | 49.761         | 49.761          | 100 NM_000154-... | 25   |
| 183.347    | 52.96          | 52.96           | 105 NM_000154-... | 27   |
| 183.839    | 59.182         | 59.182          | 106 NM_000154-... | 27   |
| 185.214    | 55.29          | 55.29           | 107 NM_000154-... | 27   |
| 173.779    | 47.325         | 47.325          | 108 NM_000154-... | 27   |
| 163.353    | 43.872         | 43.872          | 121 NM_000154-... | 31   |
| 161.341    | 46.171         | 46.171          | 122 NM_000154-... | 31   |
| 167.565    | 43.076         | 43.076          | 124 NM_000154-... | 31   |
| 166.107    | 48.002         | 48.002          | 103 NM_000154-... | 31   |

Information





# OMERO ScreenPlateWell: High Content Assays

File Edit View Window Help

Hierarchies

- Jnj Account
  - Colonies 3D - multi-page tiff [1]
  - deltavision format [1]
  - first one [1]
  - Flex 3D uncompressed 8 july [1]
  - Flex uncompressed 8 july 200
    - 12132846 384\_CellCarrie
    - 12134780 384\_CellCarrie
    - 12134812 384\_CellCarrie
    - 12134860 384\_CellCarrie
  - tumour colonies 3D flex [2]
    - 365700055641
    - 365700055641
    - Greiner uClear
    - Greiner uClear
    - Greiner uClear

Field #1

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A |   |   |   |   |   |   |   |   |   |    |    |    |
| B |   |   |   |   |   |   |   |   |   |    |    |    |
| C |   |   |   |   |   |   |   |   |   |    |    |    |
| D |   |   |   |   |   |   |   |   |   |    |    |    |
| E |   |   |   |   |   |   |   |   |   |    |    |    |
| F |   |   |   |   |   |   |   |   |   |    |    |    |

Plate's details

Plate ID: 103  
Greiner uClear

☒ private ☐ public

Annotations

rate: ★★★★★  
tag: None  
attachment: 2009\_97\_240\_IM.xdce  
Mara\_Alexa488\_and Hoechst\_Ef

comment

Comments

[ID: 3582] Well F-5, Field #1

Controls Display Zoom View Window Help

Image

Compression: High

460  
535

Z=1/1 T=1/1 x0.25

[ID: 3670] Well E-10, Field #1

Controls Display Zoom View Window Help

Image

Compression: High

460  
535

Z=1/1 T=1/1 x0.25

[ID: 3628] Well D-2, Field #1

Controls Display Zoom View Window Help

Image

Compression: High

460  
535

Z=1/1 T=1/1 x0.25

# High Content Screening Applications

- Systems
  - INCELL(GE), OPERA(Perkin Elmer), Cellomics(ThermoScientific)
- Applications
  - Cellprofiler/CellProfiler Analyst(Broad Institute)
  - Cellcognition(ETH Zurich)
  - Definiens Developer XD(Definiens)
  - Columbus(Perkin Elmer)



# OMERO – HCS – Columbus

Image Analysis - Windows Internet Explorer

http://localhost:8283/php/imageviewer/viewer.php?title=Columbus%20Navigation&pid\_id=116&session\_id=151&url\_prefix=http://localhost:8283

Favorites Suggested Sites Web Slice Gallery

Columbus Home Page Image Analysis

Columbus Columbus (columbus)

Screen List

- Acapella Script Collection
- Arrayscan
- BD Pathway
- Discovery-1
- INCell
- Opera
- Operetta
- OTP
- 2010-01-26T08:57:02Z
- P001-CT Peroxisomes
- 2009-02-03T12:43:00Z
- P010-R ETAR
- Small-z\_Stack

Input Image

Find Nuclei

Find Cytoplasm

Channel: Alexa 488

Nuclei: Nuclei

Method: 0

Individual Threshold

Define Results

Controls

Channels

Overlays

Nucleus

body

Cell

Membrane

Cytoplasm

body

Detected cells. Inspect results and if required adjust parameters.

Summary Properties Nuclei

Population- FindCytoplasm\_illustrative

Number of Objects: 60

| Property                      | Mean    | CV %    | StdDev  | Median  | Max     | Min     | Sum      |
|-------------------------------|---------|---------|---------|---------|---------|---------|----------|
| Cell Area [px²]               | 16321.7 | 55.6593 | 9084.53 | 15279.0 | 58686.0 | 1181.0  | 979300.0 |
| Cell Area [µm²]               | 1006.33 | 55.6593 | 560.115 | 938.157 | 3618.34 | 72.8157 | 60379.7  |
| Cytoplasm Area [px²]          | 12103.5 | 64.5785 | 7816.25 | 11288   | 49660   | 302     | 736209   |
| Cytoplasm Area [µm²]          | 746.252 | 64.5785 | 481.918 | 689.036 | 3061.83 | 18.6201 | 44775.1  |
| Cell Intensity Alexa 488      | 1556.04 | 12.3698 | 192.479 | 1533.17 | 2659.9  | 1132.38 | 93362.7  |
| Cytoplasm Intensity Alexa 488 | 1528.06 | 9.42536 | 144.026 | 1517.16 | 1854.46 | 1081.82 | 91683.8  |

Field

Plane

Well

Done

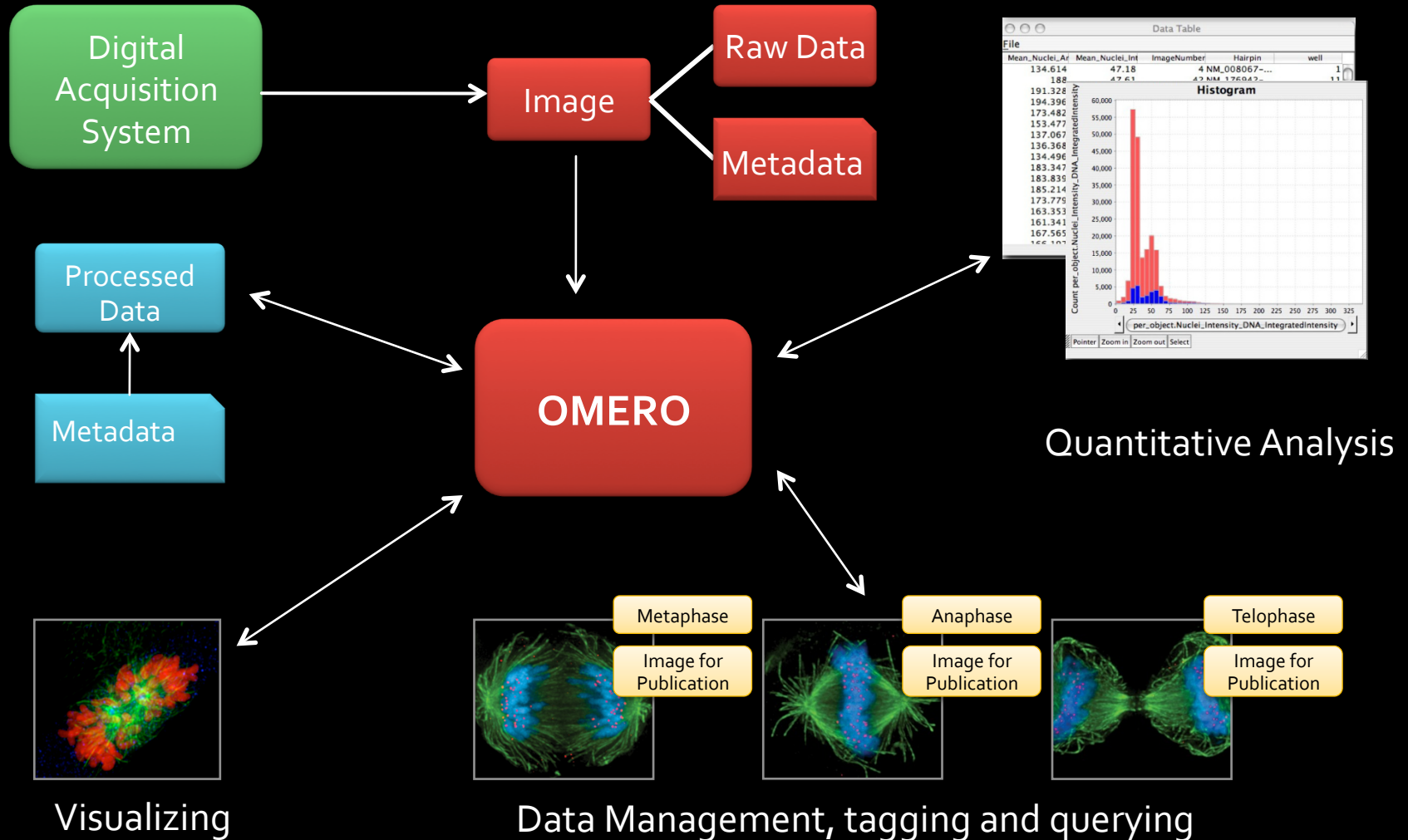
Local intranet | Protected Mode: Off

100%

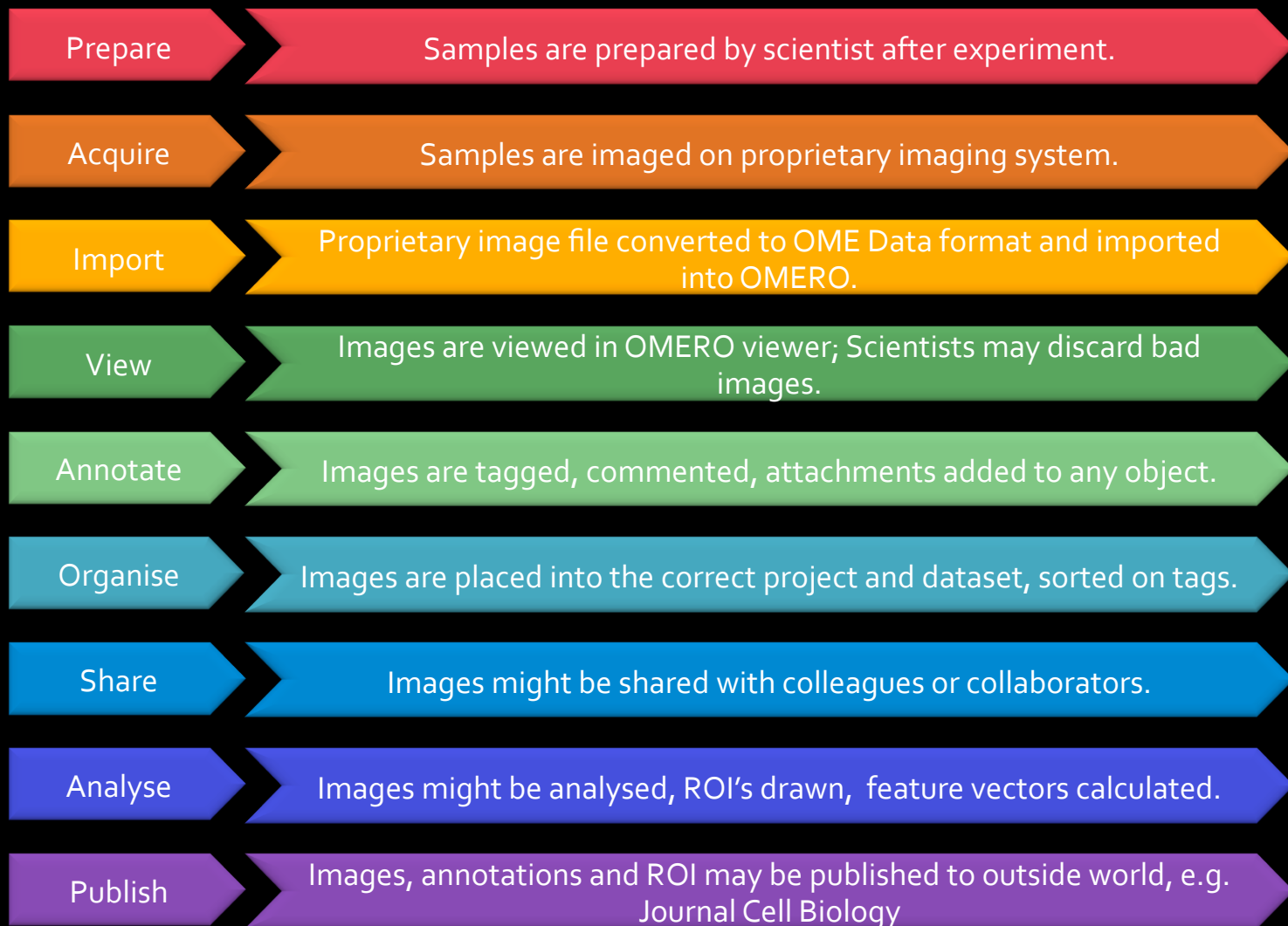
# High Content Screening - Data

- Type: 5D Images
- Data type: typically 8, 12 or 16bit
- X, Y: 512, 512 now moving on to 2048, 2048
- Z: Commonly only on 1 section
- T: 0, but recent article in science showing live cell HCS.
- C: 3-4 typical, can be more.
- Size: 1GB-60GB

# What does OMERO do?



# Imaging Workflow

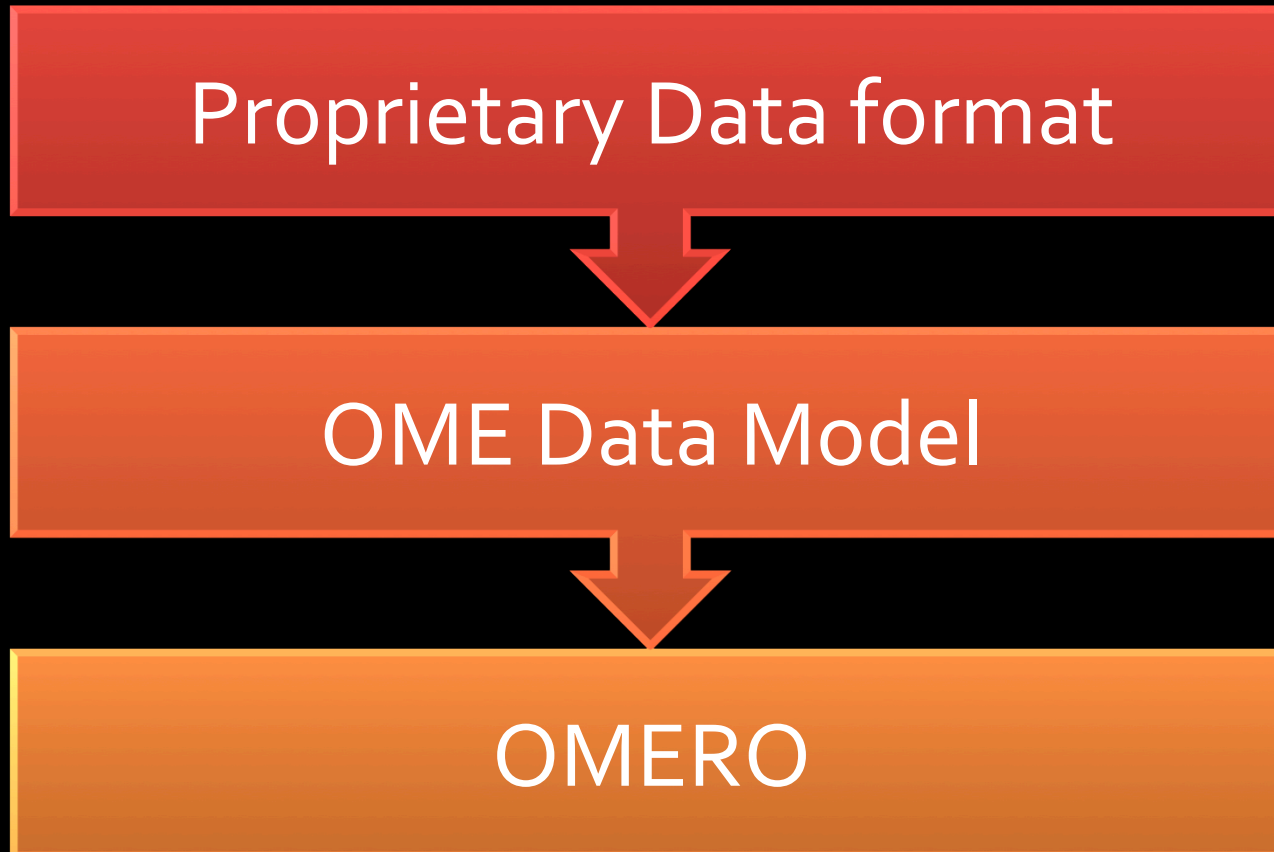


# Imaging Workflow – OMERO Applications



# The OMERO Data Model

The OME Data model unifies the metadata  
for over 100 proprietary data formats

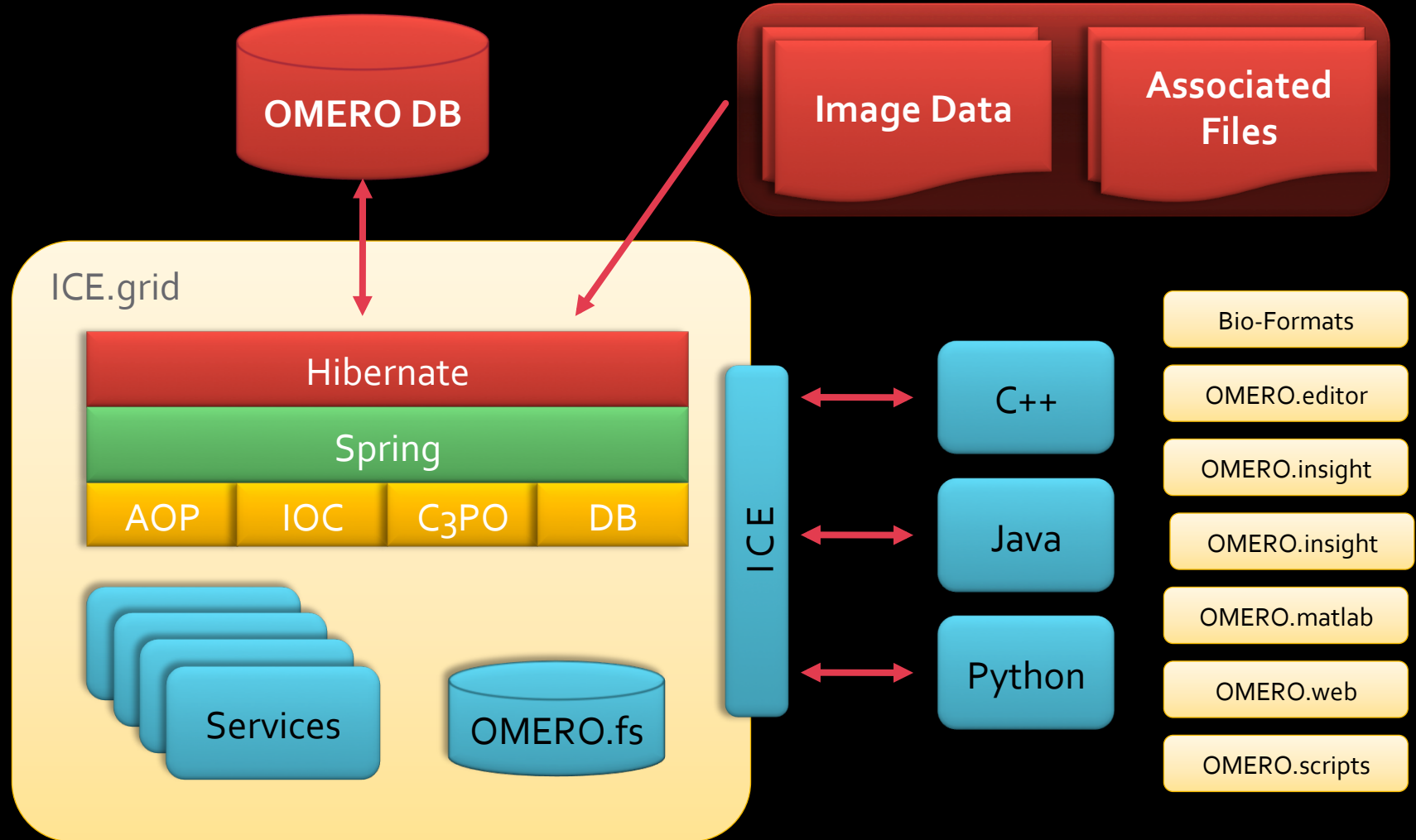


# Bioformats

---

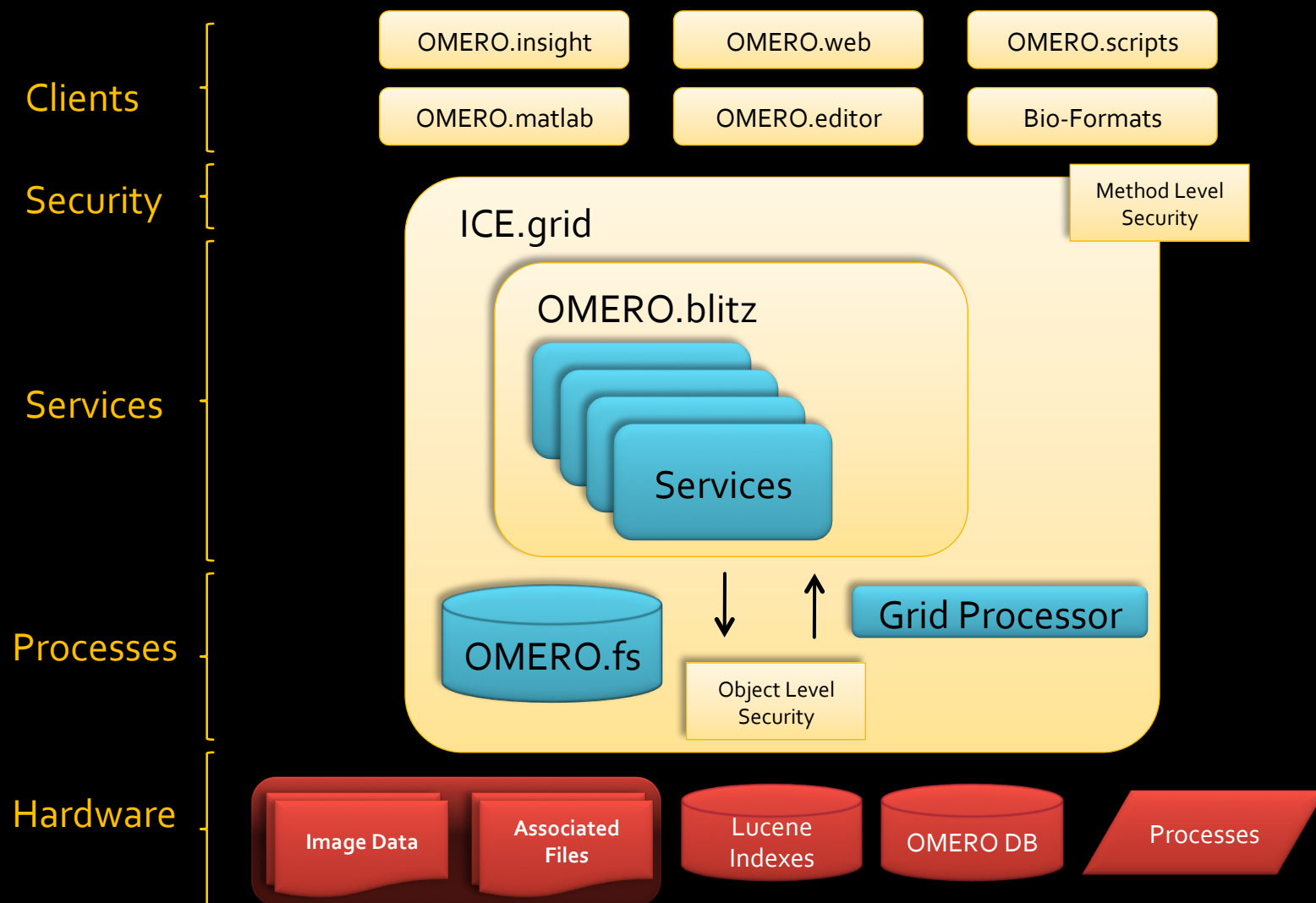
- Converts over 100 proprietary file formats to OME Data model
- Also used with ImageJ, Matlab, etc.
- Converts the image to OME-XML or OME-TIFF

# OMERO Structure





# OMERO Platform



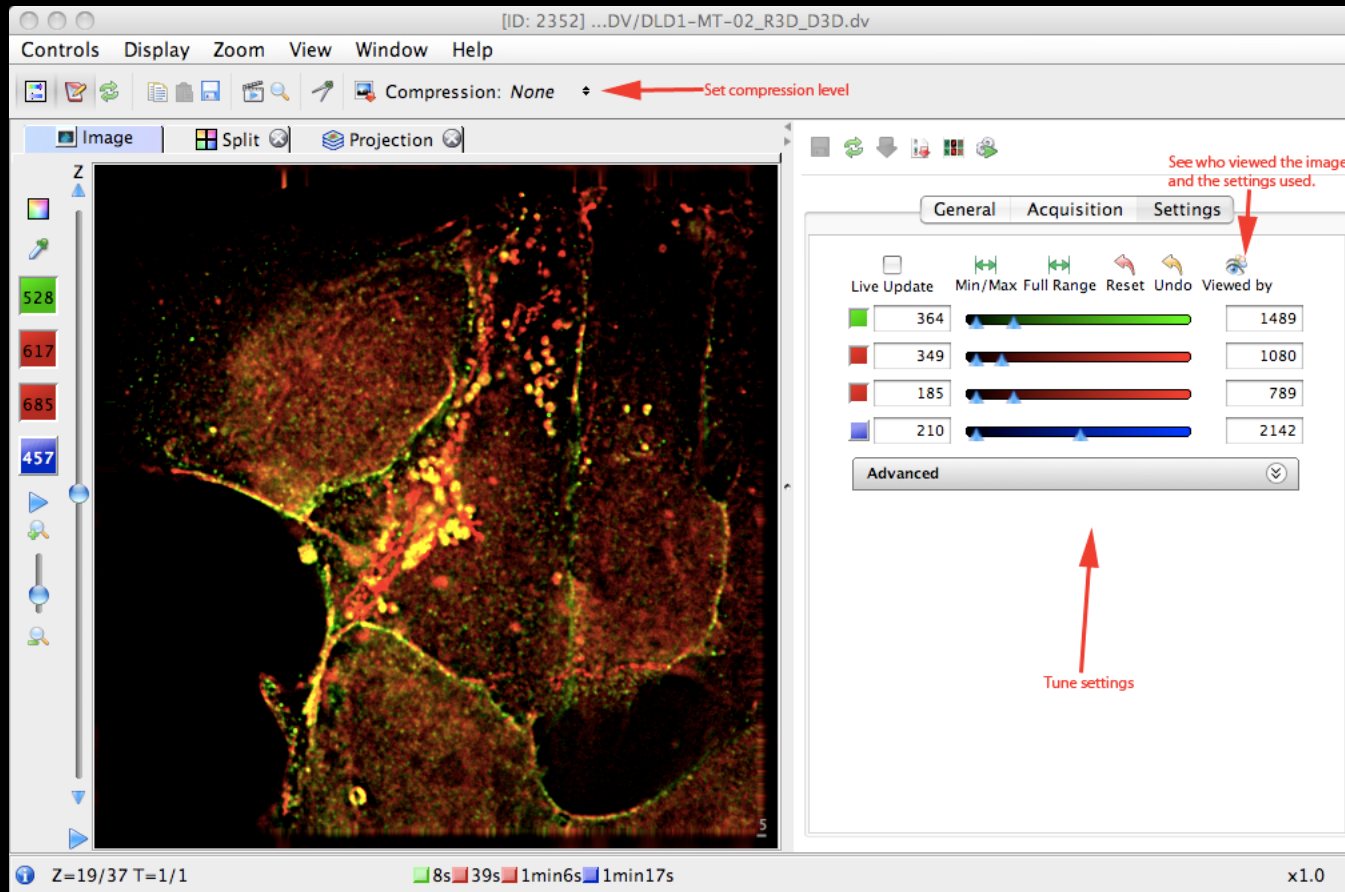
# OMERO Services

- OMERO Services are either stateless:
  - IAdmin
  - IUpdate
  - IQuery
  - IConfig
- or stateful:
  - RenderingEngine
  - RawPixelStore
  - RawFileStore
  - IProjection

# OMERO Service – Rendering Engine

- RenderingEngine
  - User defines a set of rendering settings
  - Applies the users rendering settings to the raw data
  - Returns an image to the user

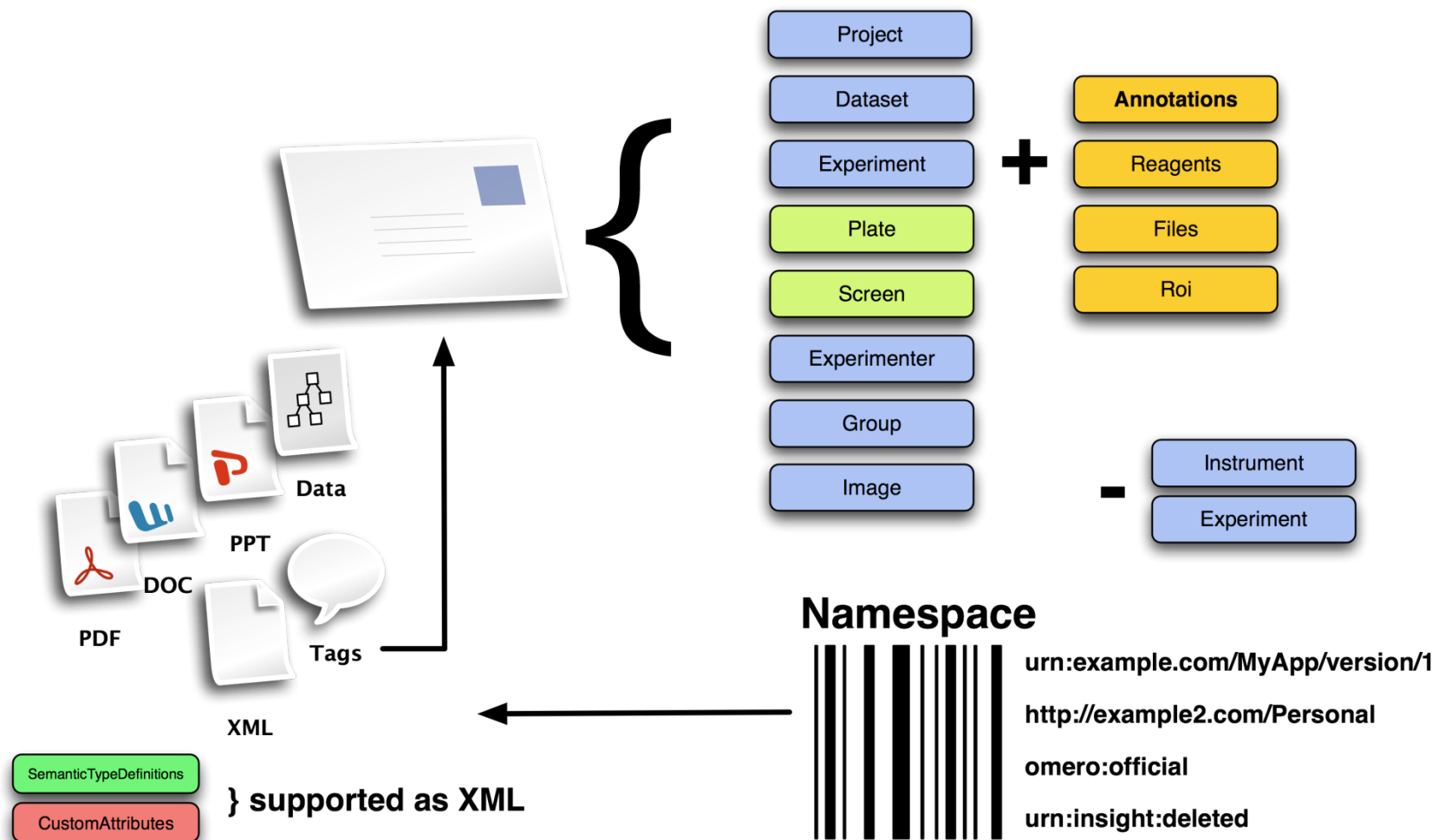
# OMERO Services



# Storing Analysis Results

- Structure your image data
- Annotate your analysis results
- Attach your analysis results
  - Images
  - Datasets
  - Projects
  - Other data

# OMERO: Structured Annotations



# OMERO: Storing Tabular Data

/OMERO/Files/1 (HDF file)

"/OME/Measurement:Plate1" (HDF group)

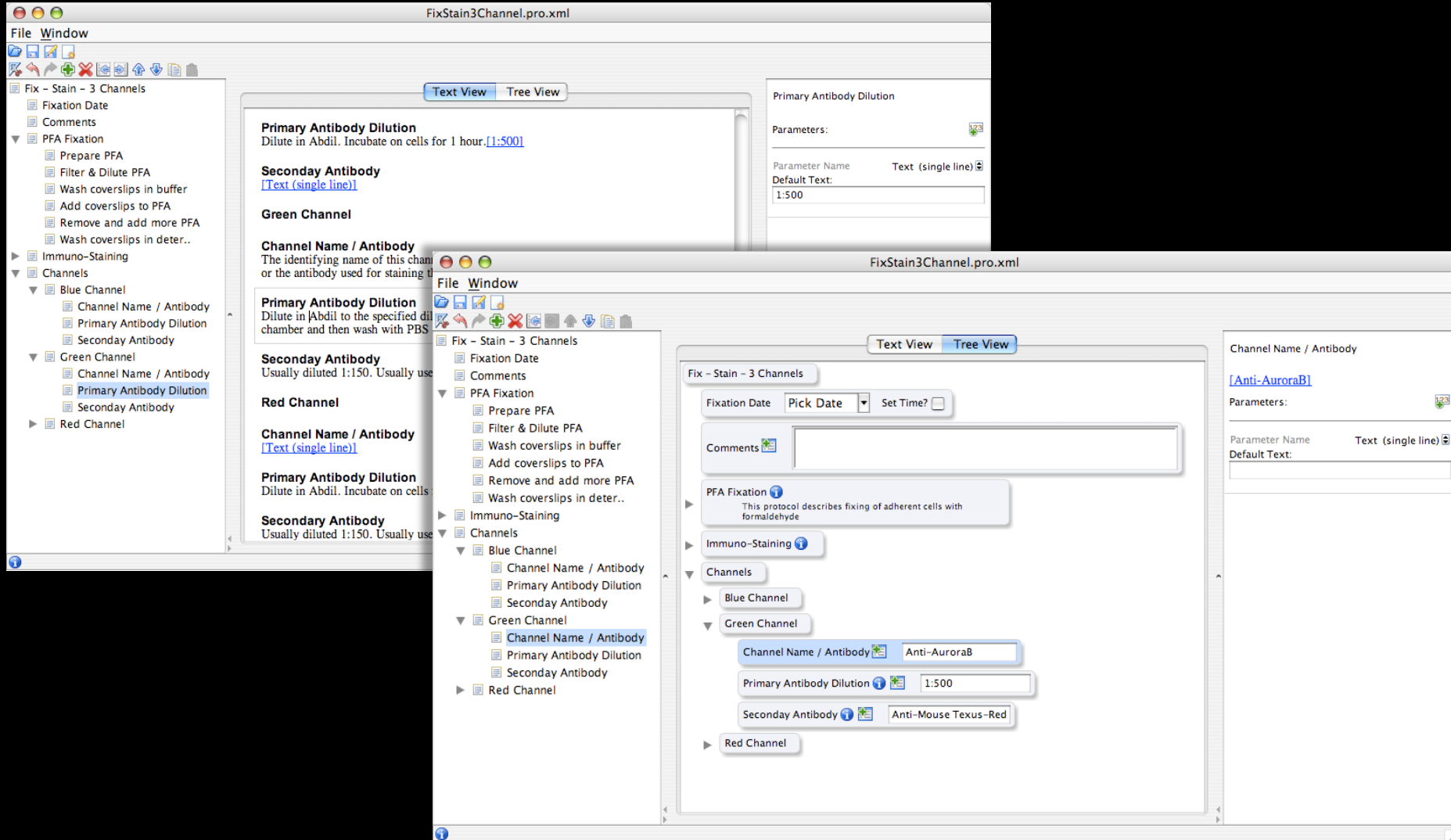
| ImageColumn | LongColumn | DoubleColumn | DoubleColumn | DoubleColumn |
|-------------|------------|--------------|--------------|--------------|
|             |            |              |              |              |

Aggregation  
(e.g. mean)

| SourceColumn | RoiColumn | DoubleColumn | DoubleColumn | DoubleColumn |
|--------------|-----------|--------------|--------------|--------------|
|              |           |              |              |              |

"/OME/Measurement:Image1" (HDF group)

# Electronic Lab Notebook – OMERO.editor

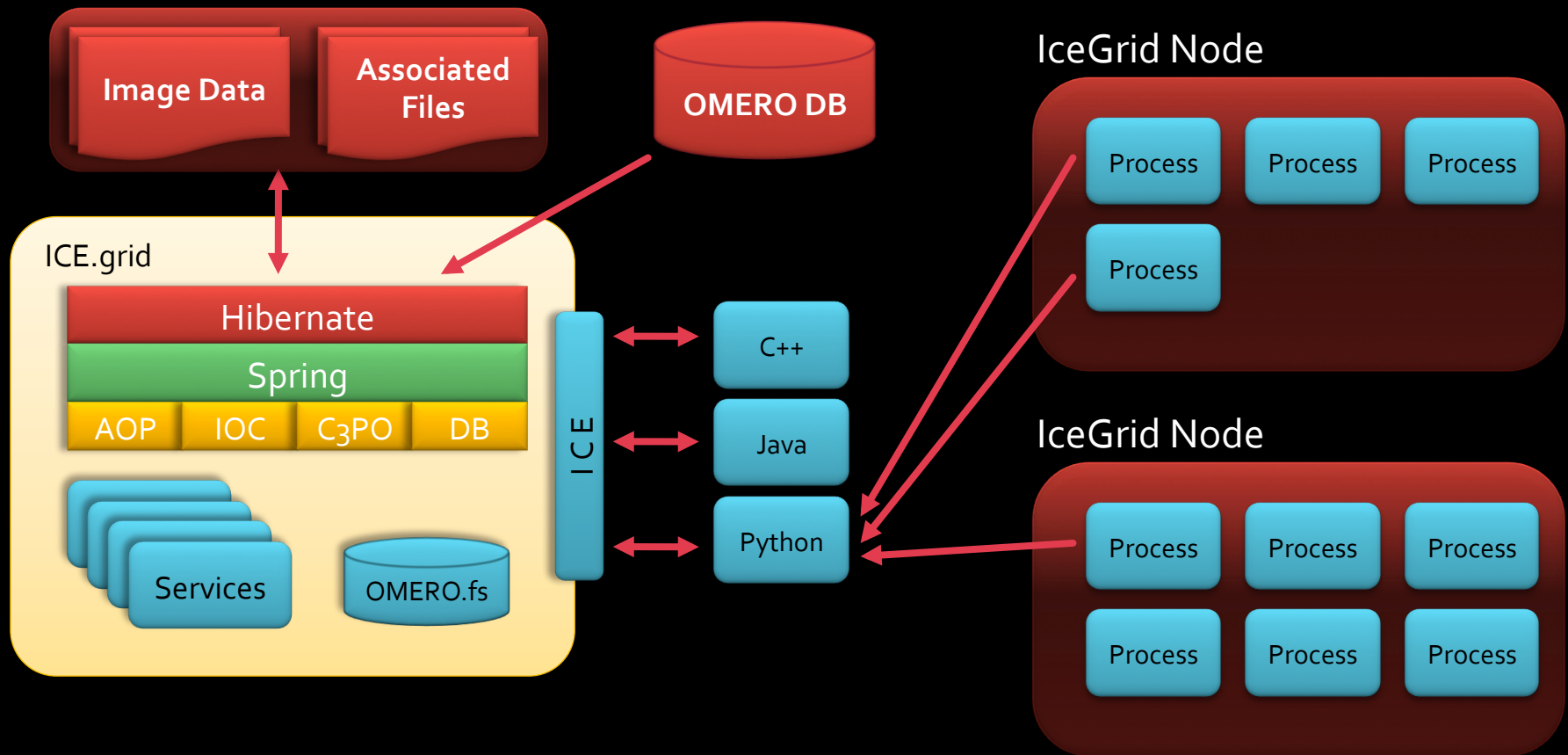




# Electronic Lab Notebook – OMERO.editor

- OMERO.editor is designed to facilitate recording of experimental metadata, for annotation of images in OMERO.
- An editing tool, where users can create a "template" (for example, to describe a protocol) and then use this template to create individual "experiment" files, which contain the experimental metadata.
- A summary of the experiment can be viewed alongside annotated images in OMERO.insight. This workflow makes it easy to reuse protocols, and to build up a detailed description of an experiment by combining several smaller protocols.
- OMERO.editor saves files as XML documents, which makes it possible for them to be read by other software.

# Distributed Processing – IceGrid

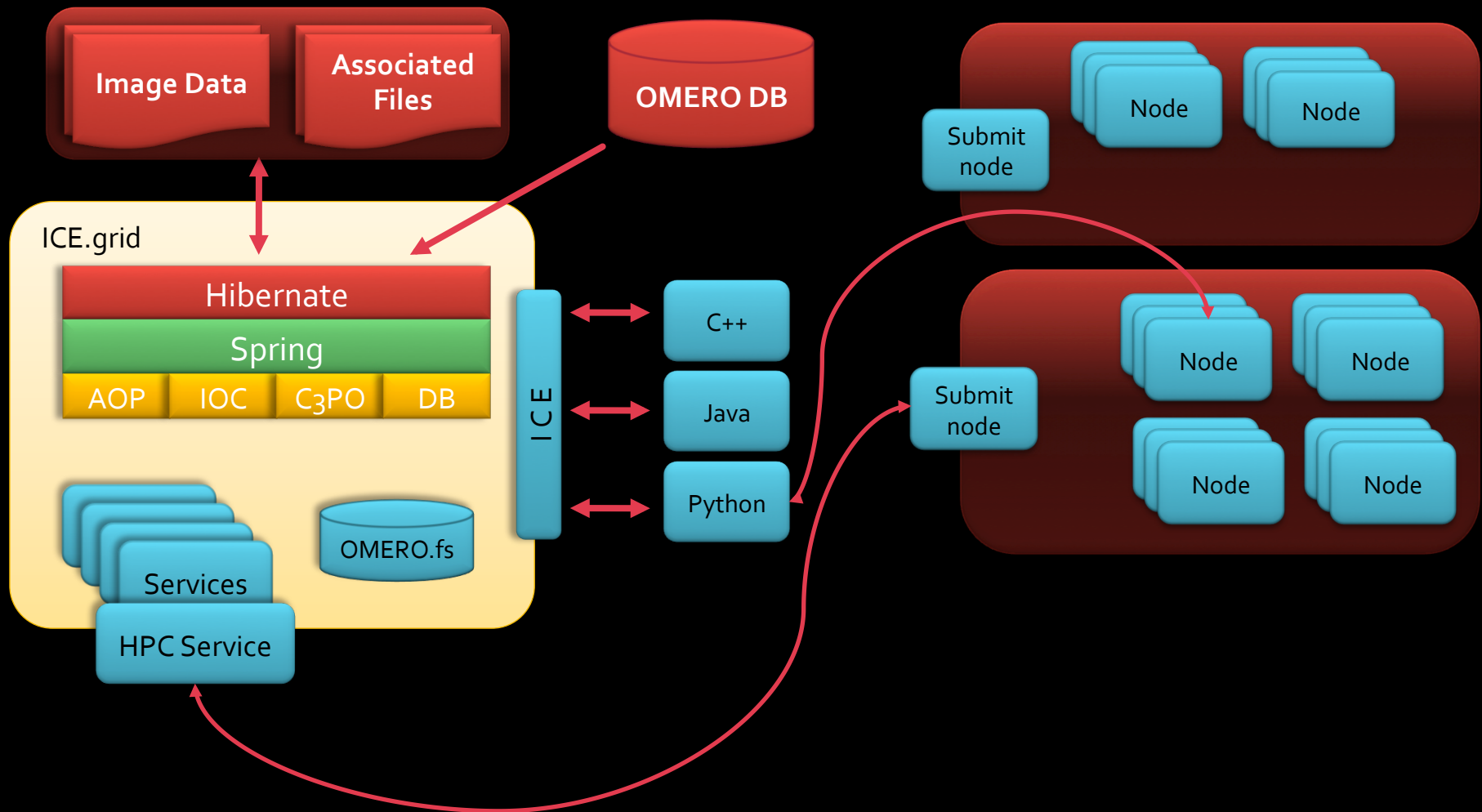


All nodes managed by IceGrid manager, like folding@home relies on installation of IceGrid node software

# IceGrid

- Pros
  - OMERO is an IceGrid Process so can deploy multiple servers easily
- Cons
  - Need to install IceGrid Process on all nodes
  - Need to define nodes at deploy time
  - Need to be IceGrid aware
  - Non-standard
  - Not a scheduling system, just runs a process
  - Copying data from OMERO to node

# Distributed Processing – RAPID



# OMERO.rapid workflow

- Xml specification held on OMERO server
- User calls HPC service (which wraps RAPID) with id of xml file and parameters.
- Rapid submits the job using the xml specification
- The resulting script runs on the cluster
  - gets the data from OMERO
    - There are two datasets, one for control and one containing the experimental condition.
  - Distributes image via MPI to worker nodes
  - The analysis results are attached to the dataset containing the images with the experimental condition

# OMERO.rapid

## ■ Pros

- Can use multiple JSE: PBS, GridEngine, Condor,...
- Need not be OMERO/ICE aware
- Configurable via XML script
- Possible to make the system OMERO agnostic

## ■ Cons

- Cluster needs to have libs
- Copying data from OMERO to node