



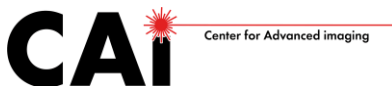
## Managing Plates in OMERO

Uploading, annotating, exploring, challenges

Anna Hamacher M.Sc.

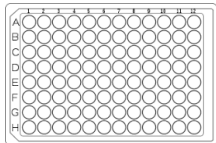
apl. Prof. Dr. Stefanie Weidtkamp-Peters

Center for Advanced Imaging



## ■ What is special about plate data?

- Additional layer



Project  
➤ Dataset  
➤ Image

vs.

Screen  
➤ Plate  
➤ Well  
➤ Image



- Many single images (typically 1k - 50k per plate)
- Equal imaging settings, but many different sample conditions
- Filename contains no metadata except position & dimension, e.g. r04c09f05p02-ch6sk1fk1fl1.tif
- Structured metadata of the plate is available before an image is created

## ■ Our data comes from a **PerkinElmer Operetta CLS HCS system**

- Expensive Harmony Software licences



# Uploading Plate Data

## 1. Via OMERO.insight

- Pretty straightforward
- Uploads full folder structure!



Name	Date modified	Name
Assaylayout	10/12/2021 14:5	Index.idx
FFC_Profile	10/12/2021 14:5	
Images	10/12/2021 14:5	

Name	Date	Type
r02c03f01p01-ch1sk1fk1f11	19/11/2021 13:08	XML Document
r02c03f01p01-ch2sk1fk1f11	19/11/2021 13:06	TIFF File
r02c03f01p01-ch3sk1fk1f11	19/11/2021 13:06	TIFF File
r02c03f01p01-ch4sk1fk1f11	19/11/2021 13:06	TIFF File
r02c03f01p01-ch5sk1fk1f11	19/11/2021 13:06	TIFF File
r02c03f02p01-ch1sk1fk1f11	19/11/2021 13:06	TIFF File
r02c03f02p01-ch2sk1fk1f11	19/11/2021 13:06	TIFF File
r02c03f02p01-ch3sk1fk1f11	19/11/2021 13:06	TIFF File
r02c03f02p01-ch4sk1fk1f11	19/11/2021 13:06	TIFF File

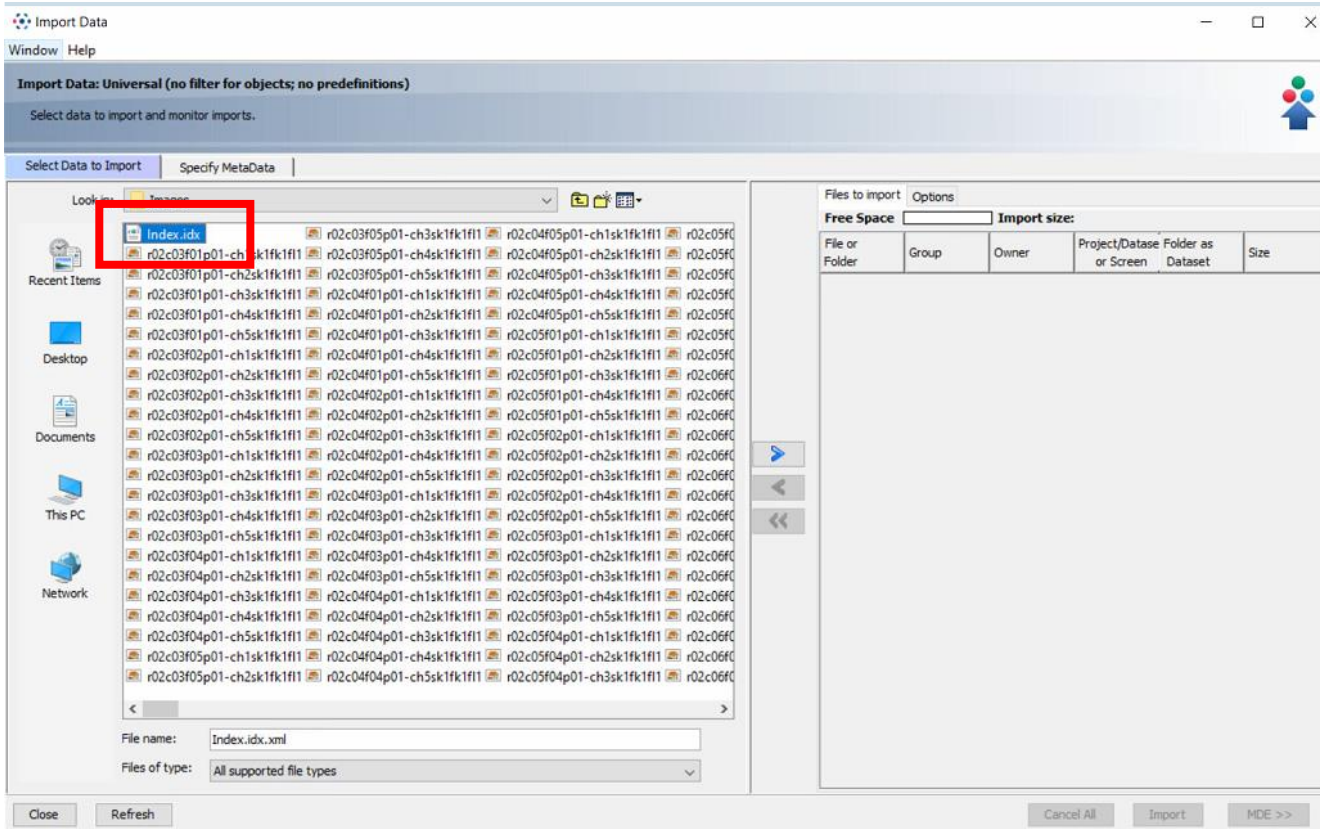


## 2. Without index file:

- Server-side-script (util\_scripts > **Dataset to Plate**)

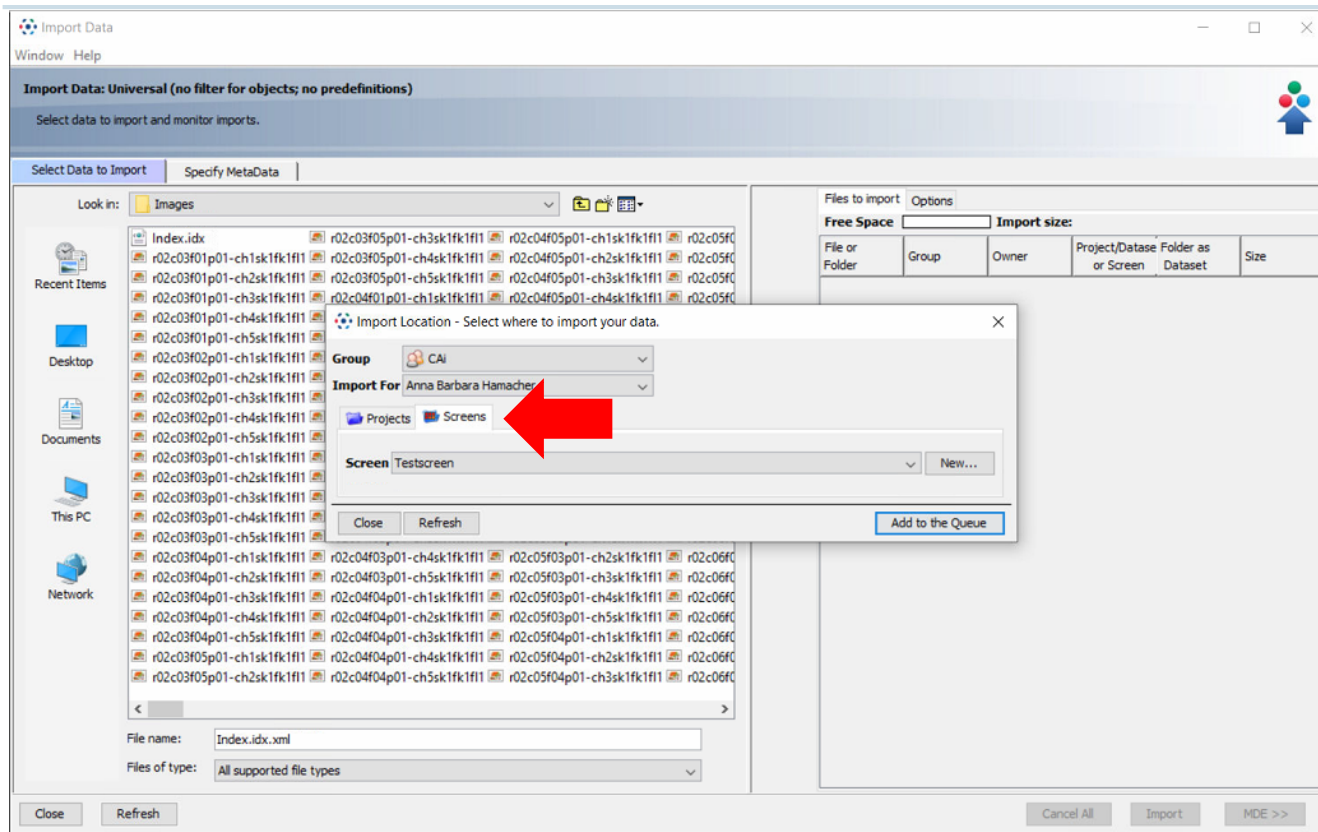
```
4149 <ObjectiveNA Unit="">0.4</ObjectiveNA>
4150 <ExposureTime Unit="s">0.04</ExposureTime>
4151 <OrientationMatrix>[[1.008174,-0.000434,0,14.2],[-0.000893,-1.0092
4152 </Image>
4153 <Image Version="1">
4154 <id>0205K1F5P1R2</id>
4155 <State>Ok</State>
4156 <URL>r02c05f05p01-ch2sk1fk1f11.tiff</URL>
4157 <Row>2</Row>
4158 <Col>5</Col>
4159 <FieldID>5</FieldID>
4160 <PlaneID>1</PlaneID>
4161 <TimepointID>0</TimepointID>
4162 <ChannelID>2</ChannelID>
4163 <FlimID>1</FlimID>
4164 <ChannelName>Brightfield</ChannelName>
4165 <ImageType>Signal</ImageType>
4166 <AcquisitionType>NipkowConfocal</AcquisitionType>
4167 <IlluminationType>Transmitted</IlluminationType>
4168 <ChannelType>Brightfield</ChannelType>
4169 <ImageResolutionX Unit="m">2.9898804047838085E-07</ImageResolution>
4170 <ImageResolutionY Unit="m">2.9898804047838085E-07</ImageResolution>
4171 <ImageSizeX>2160</ImageSizeX>
4172 <ImageSizeY>2160</ImageSizeY>
4173 <BinningX>1</BinningX>
4174 <BinningY>1</BinningY>
4175 <MaxIntensity>65535</MaxIntensity>
4176 <CameraType>AndorZylaCam</CameraType>
4177 <PositionX Unit="m">0</PositionX>
4178 <PositionY Unit="m">-0.001291628</PositionY>
4179 <PositionZ Unit="m">9E-06</PositionZ>
4180 <AbsPositionZ Unit="m">0.135086805</AbsPositionZ>
4181 <MeasurementTimeOffset Unit="s">0</MeasurementTimeOffset>
4182 <AbsTime>2021-11-19T12:56:34.153+01:00</AbsTime>
4183 <MainExcitationWavelength Unit="nm">740</MainExcitationWavelength>
4184 <MainEmissionWavelength Unit="nm">0</MainEmissionWavelength>
4185 <ObjectiveMagnification Unit="">20</ObjectiveMagnification>
4186 <ObjectiveNA Unit="">0.4</ObjectiveNA>
4187 <ExposureTime Unit="s">0.2</ExposureTime>
4188 <OrientationMatrix>[[1.008174,-0.000434,0,14.2],[-0.000893,-1.0092
4189 </Image>
4190 <Image Version="1">
4191 <id>0205K1F5P1R3</id>
4192 <State>Ok</State>
4193 <URL>r02c05f05p01-ch3sk1fk1f11.tiff</URL>
```

# Uploading Plate Data



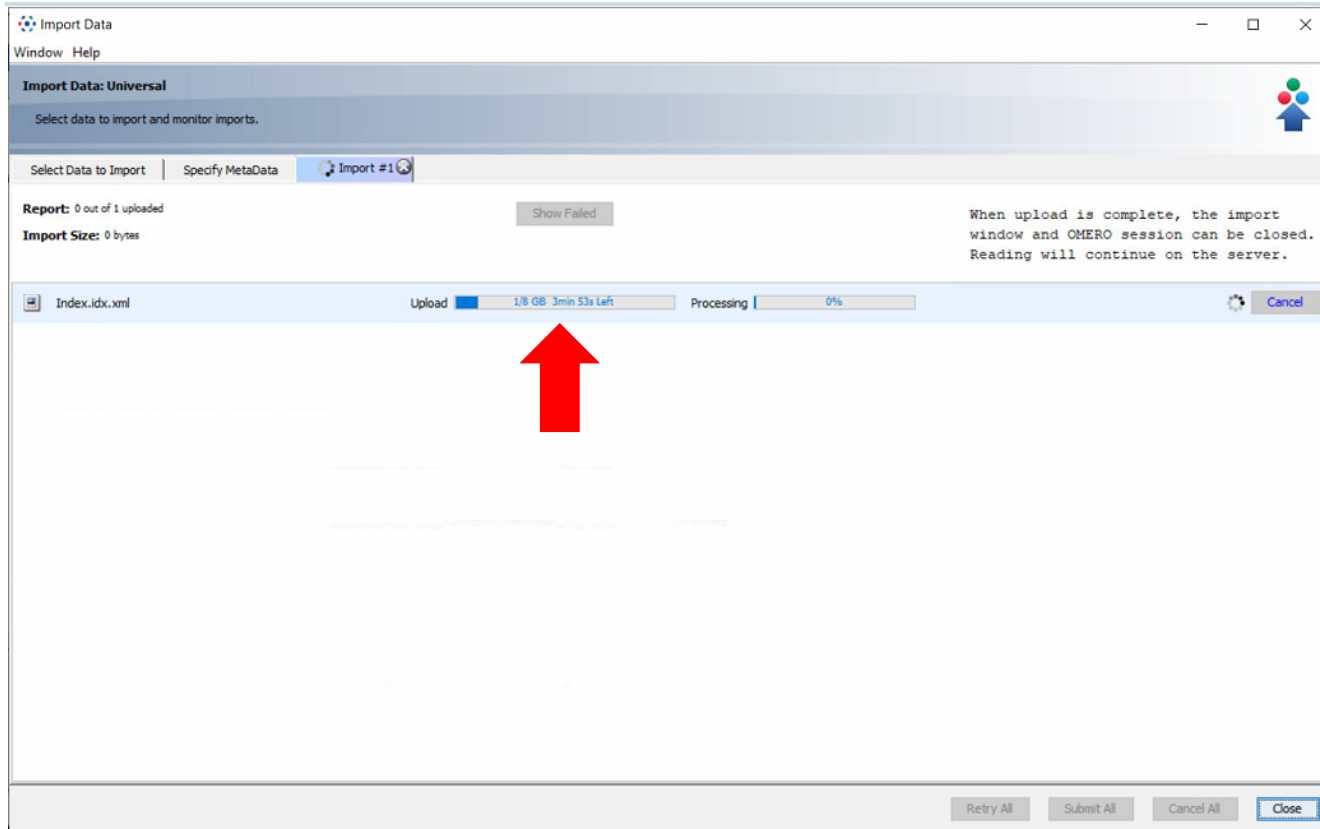
- Start insight
- Start „Importer“
- Select index file

# Uploading Plate Data



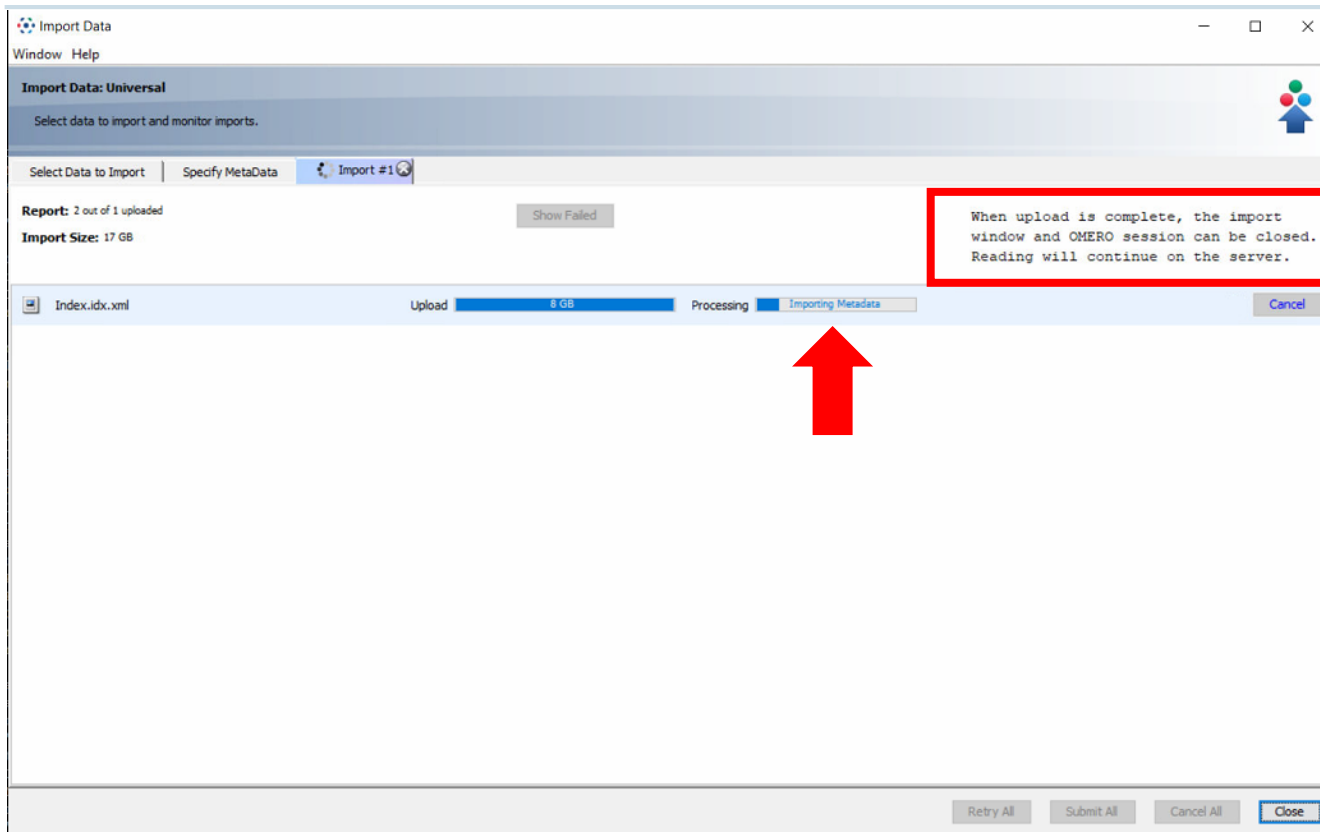
- Choose tab „Screen“
- Add to queue

# Uploading Plate Data



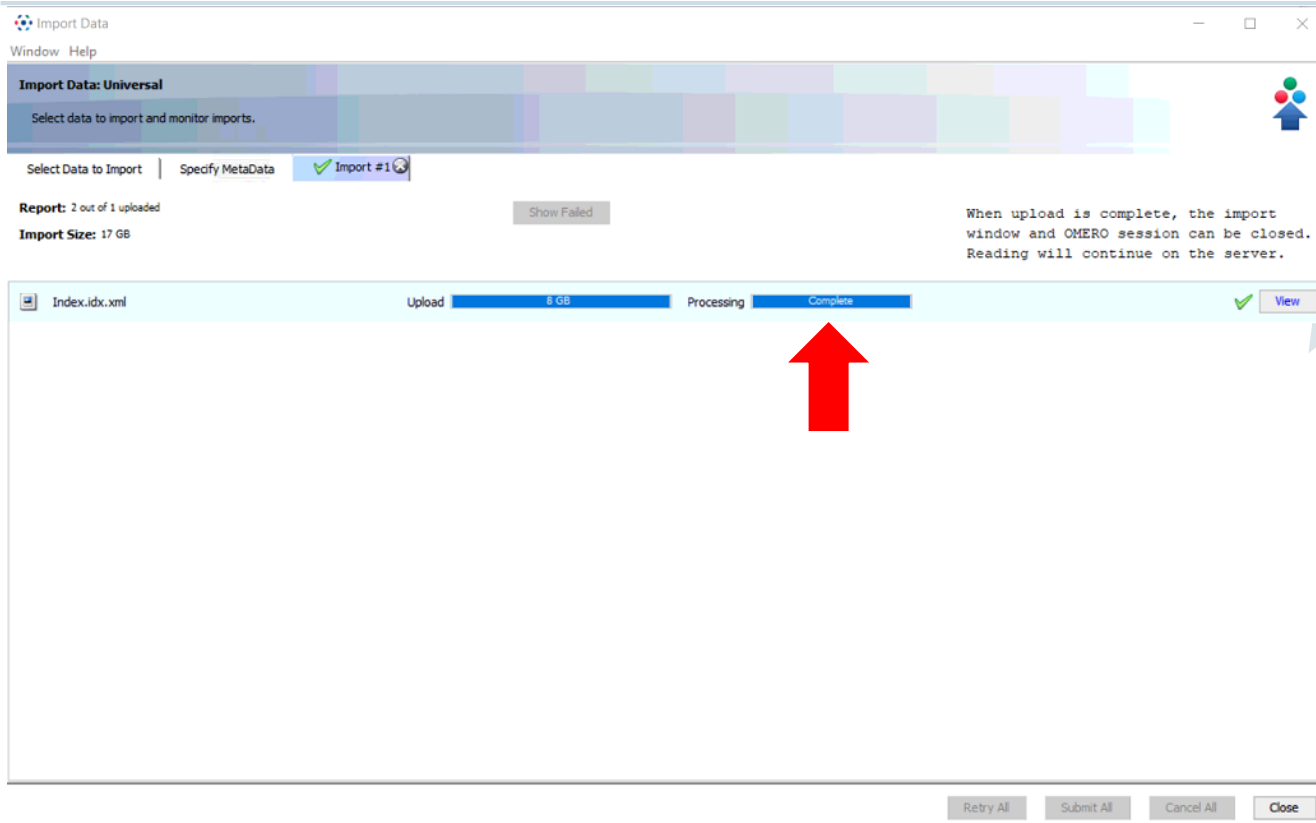
- File upload starts
- Speed depends on connection

# Uploading Plate Data



- Metadata import
- Reading pixels
- Can take multiple hours

# Uploading Plate Data



The screenshot shows the 'Import Data' window in Omero. The window title is 'Import Data' with 'Window' and 'Help' menus. Below the title bar, there's a section 'Import Data: Universal' with the instruction 'Select data to import and monitor imports.' and a button 'Import #1' with a green checkmark. Below this, it says 'Report: 2 out of 1 uploaded' and 'Import Size: 17 GB'. A 'Show Failed' button is also present. The main area shows a progress bar for 'Index.idx.xml' with three stages: 'Upload' (8 GB), 'Processing', and 'Complete'. A red arrow points to the 'Complete' stage. To the right of the progress bar, there's a green checkmark and a 'View' button. At the bottom, there are buttons for 'Retry All', 'Submit All', 'Cancel All', and 'Close'. A text box on the right side of the window says: 'When upload is complete, the import window and Omero session can be closed. Reading will continue on the server.'

■ Done!

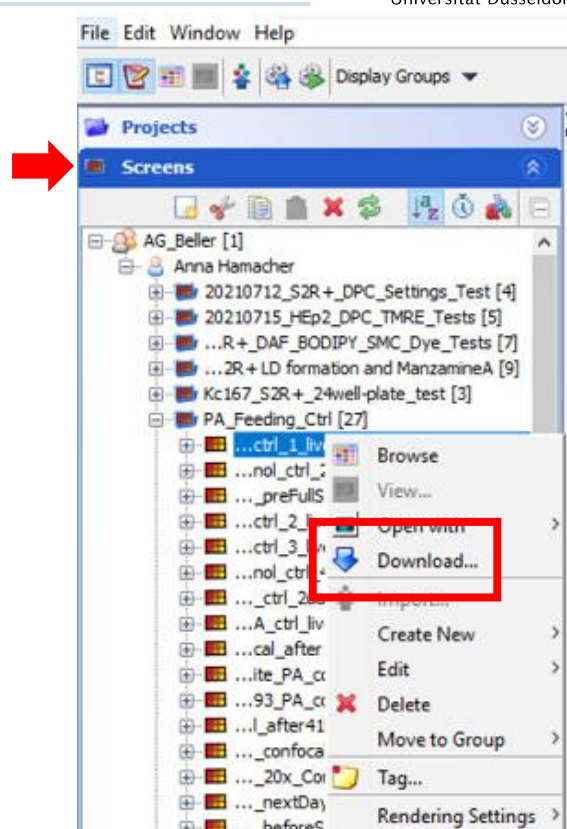
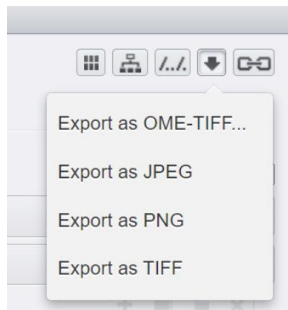
(if fails, check the log)



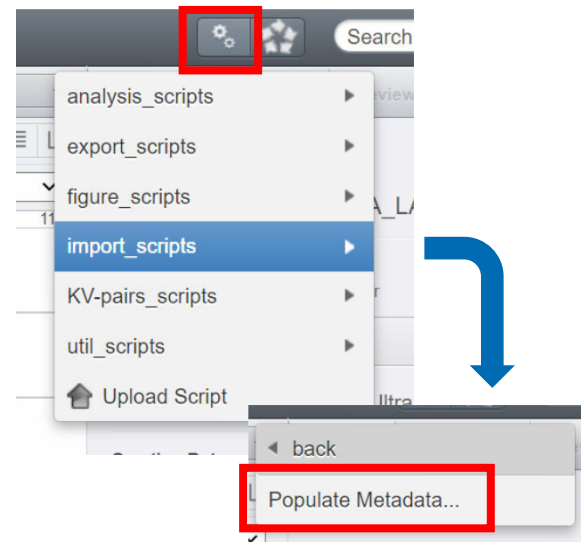
# Downloading Plate Data

- Has to be activated in the OMERO server configuration! (plate downloads are **disabled by default**)
- Only possible via OMERO.insight
- Restores full directory structure with all previous content (nice but users need to be aware of this!)
- Single image views can be exported via OMERO.web, but not the original data

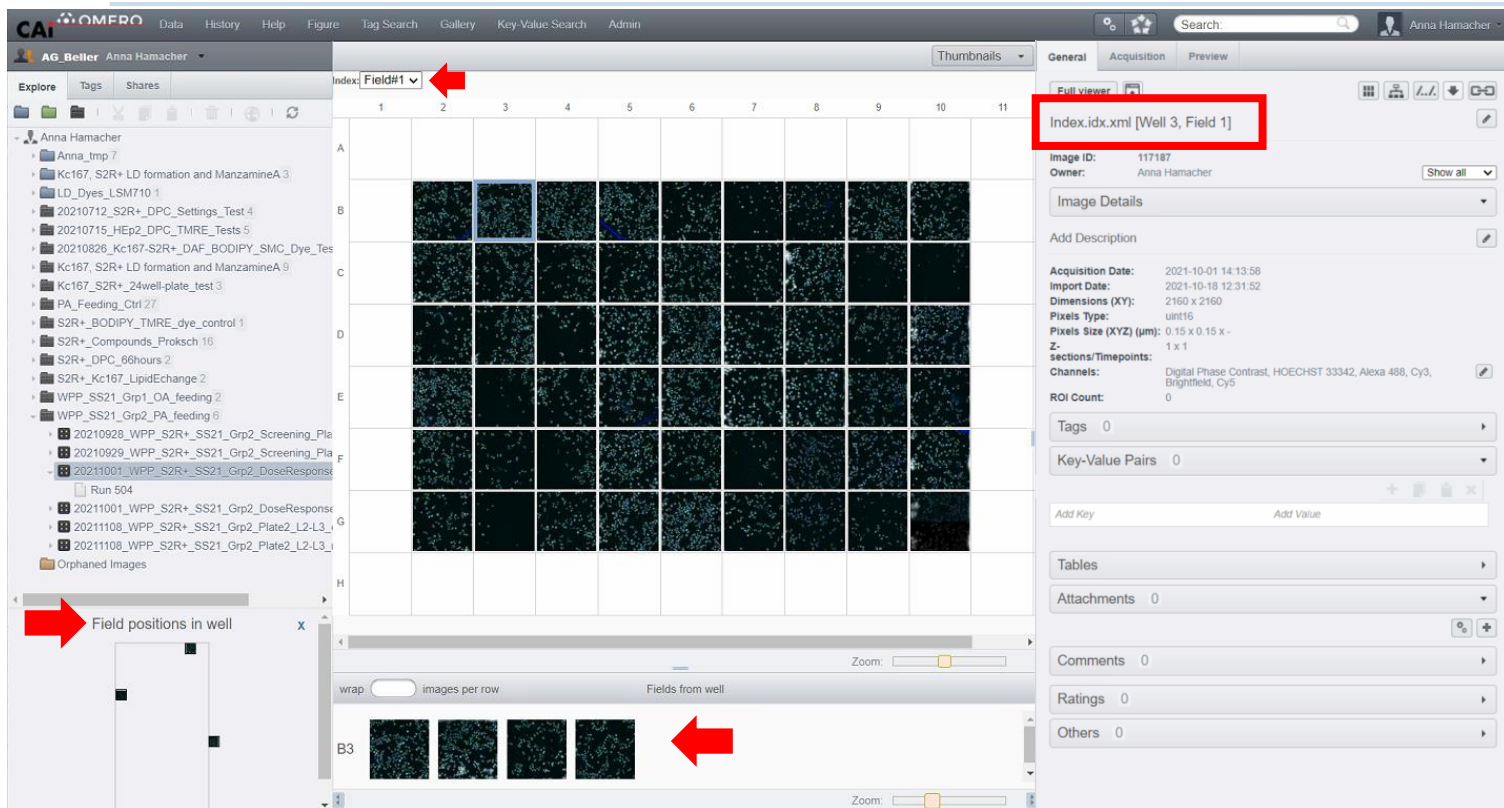
Name	Date modified	Type
Assaylayout	10/12/2021 14:56	File folder
FFC_Profile	10/12/2021 14:56	File folder
Images	10/12/2021 14:56	File folder



- **Annotate wells or images** or both? And what about plates?
  - Metadata for wells (but needs to be visible when viewing images!)
  - Image analysis results for images (but also aggregated for wells!)
  - Metadata for plates (microscope settings)
- **How?**
  - Manually (via webclient or OMERO.mde per image/image group)
  - Key-Value-Pair import via CSV-files (equivalent to implementation for Project/Dataset/Images)
  - IDR workflow (‘populate metadata’ to create a bulk annotation OMERO.table, define mapping and import als key-value-pairs)
  - Details discussed at [image.sc](https://image.sc)



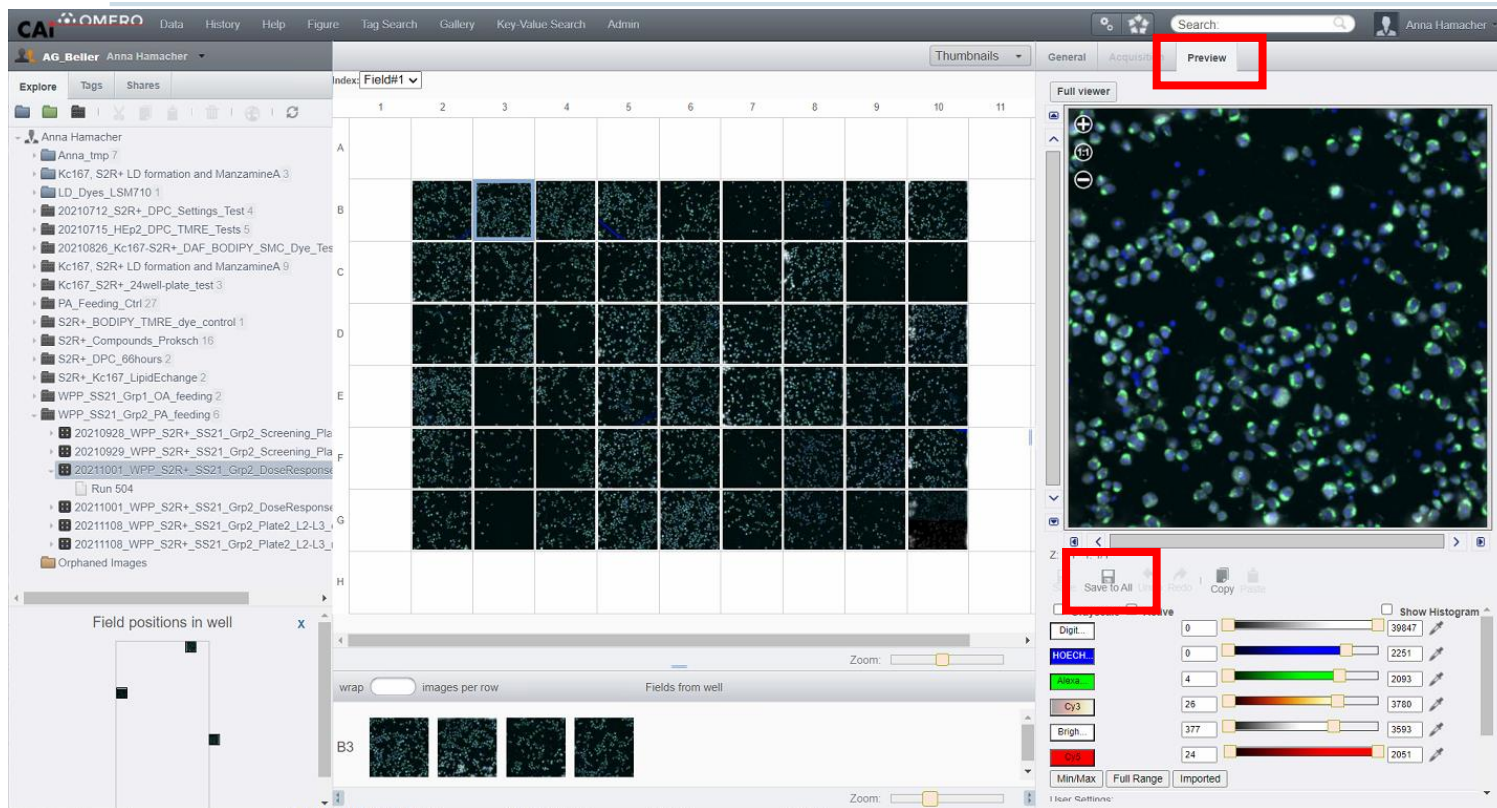
# Viewing Plate Data



The screenshot displays the CAi OMERO web interface. The top navigation bar includes links for Data, History, Help, Figure, Tag Search, Gallery, Key-Value Search, and Admin. The left sidebar shows a file tree under 'Anna Hamacher'. The main area features a grid of image thumbnails, with a dropdown menu labeled 'Index: Field#1' and a red arrow pointing to it. The right panel shows the 'General' tab with metadata for 'Index.idx.xml [Well 3, Field 1]', including acquisition date, dimensions, and channels. A red arrow points to the 'Field positions in well' dialog box at the bottom left, which shows a grid of field positions. Another red arrow points to the 'Index.idx.xml [Well 3, Field 1]' label in the right panel.


- Overview
- Plate layout
- Thumbnails
- Fields
- Positions
- Metadata

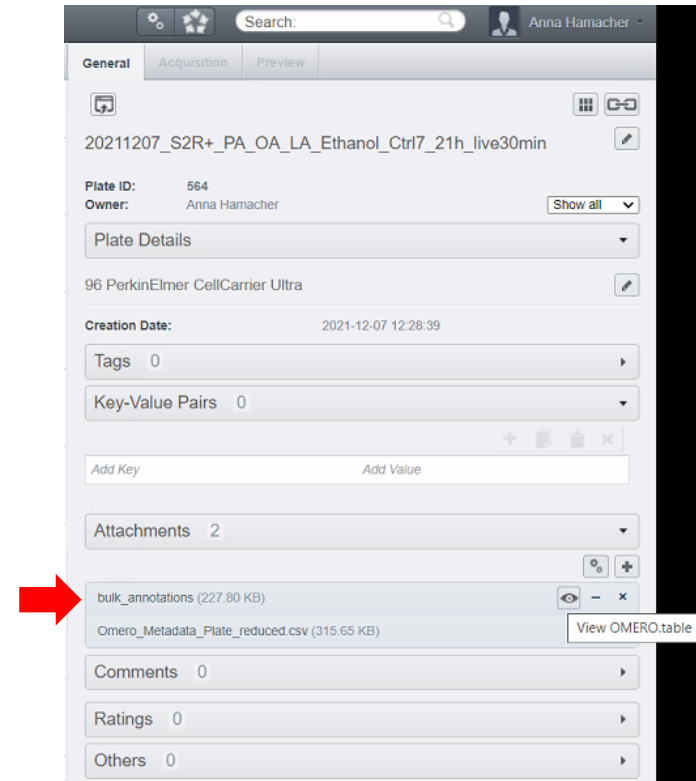
# Viewing Plate Data



- Standard UI
- Modify viewer settings
  - Channels
  - Colors
  - Dimension
  - ...
- „Save to All“ to apply on plate
- How about related data?

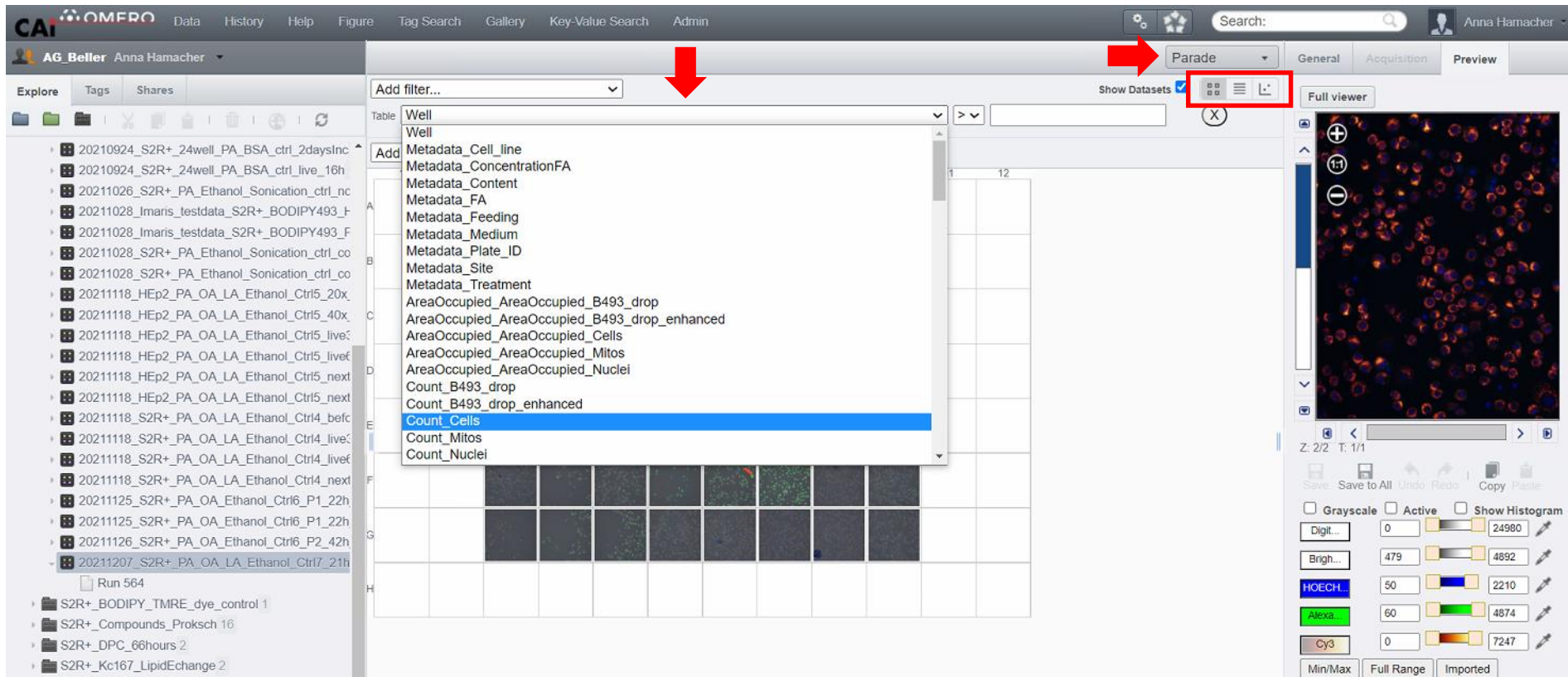
# Exploring Plate Data

- Metadata-mining plugin **OMERO.parade**
  - Plot, display and filter images based on metadata
  - Supports ROIs, Key-Value pairs and data stored in OMERO.tables
  - More infos: <https://omero-guides.readthedocs.io/>
- **Our workflow**
  - Extract analysis data from  CellProfiler™ cell image analysis software result tables
  - Prepare datasheet (CSV) with appropriate header
  - Upload CSV with `populate metadata` script to get an OMERO.tables object





# Get into OMERO.parade



The screenshot displays the OMERO.parade web interface. The top navigation bar includes links for Data, History, Help, Figure, Tag Search, Gallery, Key-Value Search, and Admin. The user is logged in as Anna Hamacher. The left sidebar shows a list of datasets, with the selected dataset being '202111207\_S2R+\_PA\_OA\_LA\_Ethanol\_Ctrl7\_21h'. The main area shows a table of metadata for the selected dataset, with a dropdown menu open for 'Add filter...'. The dropdown menu lists various metadata fields, including 'Count\_Cells', which is highlighted. A red arrow points to the 'Parade' button in the top right corner. The right sidebar shows a full viewer of the dataset, displaying a grid of microscopy images. The bottom right panel shows the 'Full viewer' controls, including a 'Z: 2/2 T: 1/1' indicator and a 'Show Histogram' checkbox.

CAI OMERO Data History Help Figure Tag Search Gallery Key-Value Search Admin

AG Beller Anna Hamacher

Explore Tags Shares

Add filter...

Table: Well

Well

Add

Metadata\_Cell\_line

Metadata\_ConcentrationFA

Metadata\_Content

Metadata\_FA

Metadata\_Feeding

Metadata\_Medium

Metadata\_Plate\_ID

Metadata\_Site

Metadata\_Treatment

AreaOccupied\_AreaOccupied\_B493\_drop

AreaOccupied\_AreaOccupied\_B493\_drop\_enhanced

AreaOccupied\_AreaOccupied\_Cells

AreaOccupied\_AreaOccupied\_Mitos

AreaOccupied\_AreaOccupied\_Nuclei

Count\_B493\_drop

Count\_B493\_drop\_enhanced

Count\_Cells

Count\_Mitos

Count\_Nuclei

Parade

Show Datasets

Full viewer

Z: 2/2 T: 1/1

Save Save to All Undo Redo Copy Paste

Grayscale Active Show Histogram

Digit... 0 24980

Bright... 479 4892

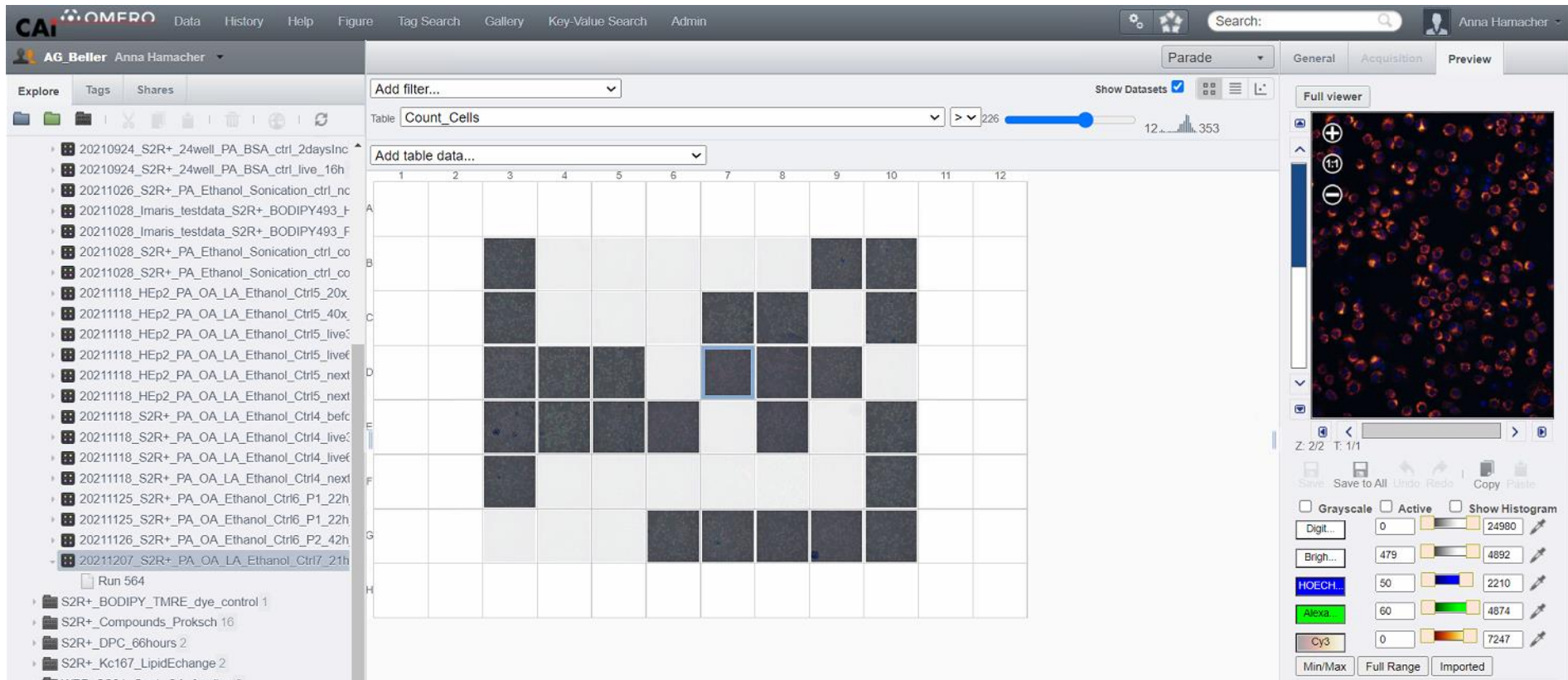
HOECHL 50 2210

Alexa 60 4874

Cy3 0 7247

Min/Max Full Range Imported

# Filtering in OMERO.parade



The screenshot displays the OMERO.parade web interface. The top navigation bar includes links for Data, History, Help, Figure, Tag Search, Gallery, Key-Value Search, and Admin. A search bar is located on the right. The left sidebar shows a list of datasets, with the selected dataset being "20211207\_S2R+\_PA\_OA\_LA\_Ethanol\_Ctrif7\_21h". The main area shows a table titled "Count\_Cells" with columns 1 through 12. A filter is applied to the table, resulting in a grid of cells. The cell at row D, column 7 is highlighted. The right sidebar shows a "Full viewer" of a microscopy image, with a histogram and color scale for the image. The histogram shows a range from 0 to 24980. The color scale is set to "Grayscale" and "Active". The image is displayed in a "Full viewer" mode.

CAI OMERO Data History Help Figure Tag Search Gallery Key-Value Search Admin

AG\_Beller Anna Hamacher

Explore Tags Shares

Add filter...

Table: Count\_Cells

Add table data...

Show Datasets [X] [Y] [Z]

12 226 353

1 2 3 4 5 6 7 8 9 10 11 12

A

B

C

D

E

F

G

H

Full viewer

Save Save to All Undo Redo Copy Paste

Grayscale Active Show Histogram

Digit... 0 24980

Bright... 479 4892

HOECH... 50 2210

Alexa... 60 4874

Cy3 0 7247

Min/Max Full Range Imported

# Table view in OMERO.parade

The screenshot displays the OMERO.parade application interface. On the left, the 'Explore' panel shows a tree view of datasets. The main area is the 'Parade' view, which is currently showing a list of datasets. A red box highlights the 'Add filter...' button in the top right corner of the Parade view. A 'Show Datasets' checkbox is also visible.

The 'Add table data...' dialog box is open, showing a list of tables to be added. The selected table is 'Table\_Mean\_Cells\_AreaShape\_Area'. The list includes various tables related to cell analysis, such as 'Table\_Mean\_B493\_drop\_enhanced\_AreaShape\_Area', 'Table\_Mean\_B493\_drop\_enhanced\_AreaShape\_Eccentricity', 'Table\_Mean\_B493\_drop\_enhanced\_AreaShape\_MeanRadius', 'Table\_Mean\_B493\_drop\_enhanced\_Distance\_Centroid\_Cells', 'Table\_Mean\_B493\_drop\_enhanced\_Intensity\_MeanIntensity\_BODIPY493', 'Table\_Mean\_B493\_drop\_enhanced\_Intensity\_MedianIntensity\_BODIPY493', 'Table\_Mean\_B493\_drop\_enhanced\_Intensity\_StdIntensity\_BODIPY493', 'Table\_Mean\_Cells\_AreaShape\_Area', 'Table\_Mean\_Cells\_AreaShape\_Eccentricity', 'Table\_Mean\_Cells\_AreaShape\_MaximumRadius', 'Table\_Mean\_Cells\_AreaShape\_MeanRadius', 'Table\_Mean\_Cells\_Children\_B493\_drop\_Count', 'Table\_Mean\_Cells\_Children\_B493\_drop\_enhanced\_Count', 'Table\_Mean\_Cells\_Children\_Mitos\_Count', 'Table\_Mean\_Cells\_Children\_Nuclei\_Count', 'Table\_Mean\_Cells\_Intensity\_MaxIntensity\_BODIPY493', 'Table\_Mean\_Cells\_Intensity\_MaxIntensity\_Hoechst', 'Table\_Mean\_Cells\_Intensity\_MaxIntensity\_TMRE', 'Table\_Mean\_Cells\_Intensity\_MeanIntensity\_BODIPY493', and 'Table\_Mean\_Cells\_Intensity\_MeanIntensity\_Hoechst'.

Index.idx.xml	Well	Field	S2R+	119.59503749999999	200	3.895	8.025526755852843	0.396766092970348
Index.idx.xml	[Well 6, Field 1]	S2R+	130.9639620535714	224	6.776785714285714	2.665866336633663	0.284753133610595	
Index.idx.xml	[Well 7, Field 1]	S2R+	122.88412322274881	211	6.696682464454977	2.6527308626974486	0.286712184093136	
Index.idx.xml	[Well 8, Field 1]	S2R+	110.6833613445378	238	3.3823529411764706	5.906619830328737	0.362809987428578	
Index.idx.xml	[Well 9, Field 1]	S2R+	112.22420600858369	233	3.4163090128755367	8.616496191512514	0.411114266631849	
Index.idx.xml	[Well 10, Field 1]	S2R+						



# Table view in OMERO.parade

CAI OMERO Data History Help Figure Tag Search Gallery Key-Value Search Admin






AG Beller Anna Hamacher

Explore Tags Shares

Add filter...

Table: Count\_Cells > 286 12 353

Add table data...

Name	Table_Metadata_Cell_line	Table_Mean_Cells_AreaShape_Area	Table_Count_Cells	Table_Mean_Cells_Children_B493_drop_Count	Table_Mean_B493_drop_AreaShape_Area	Table_Mean_B493_drop_AreaShape_
 Index.idx.xml [Well 17, Field 1]	S2R+	123.41943675889327	253	7.3470200000000015	3.8079038370440546	0.3200773596732077
 Index.idx.xml [Well 22, Field 1]	S2R+	115.80258620689654	261	6.32587043808426116	0.6326830434732609	0.1908550717371086
 Index.idx.xml [Well 25, Field 1]	S2R+	118.16778169014084	284	6.807042253921127	3.277259529553099	0.3029062874826261
 Index.idx.xml [Well 40, Field 1]	S2R+	111.55516981132074	265	6.211320754716801	3.6087526680899476	0.3160082444674294
 Index.idx.xml [Well 45, Field 1]	S2R+	130.1620817843866	269	7.048327137506406	3.0000000000000004	0.2570986842600193

Run 564

S2R+\_BODIPY\_TMRE\_dye\_control 1

S2R+\_Compounds\_Proksch 16

S2R+\_DPC\_66hours 2

S2R+\_Kc167\_LipidExchange 2

WPP\_SS21\_Grp1\_OA\_feeding 2

WPP\_SS21\_Grp2\_PA\_feeding 6

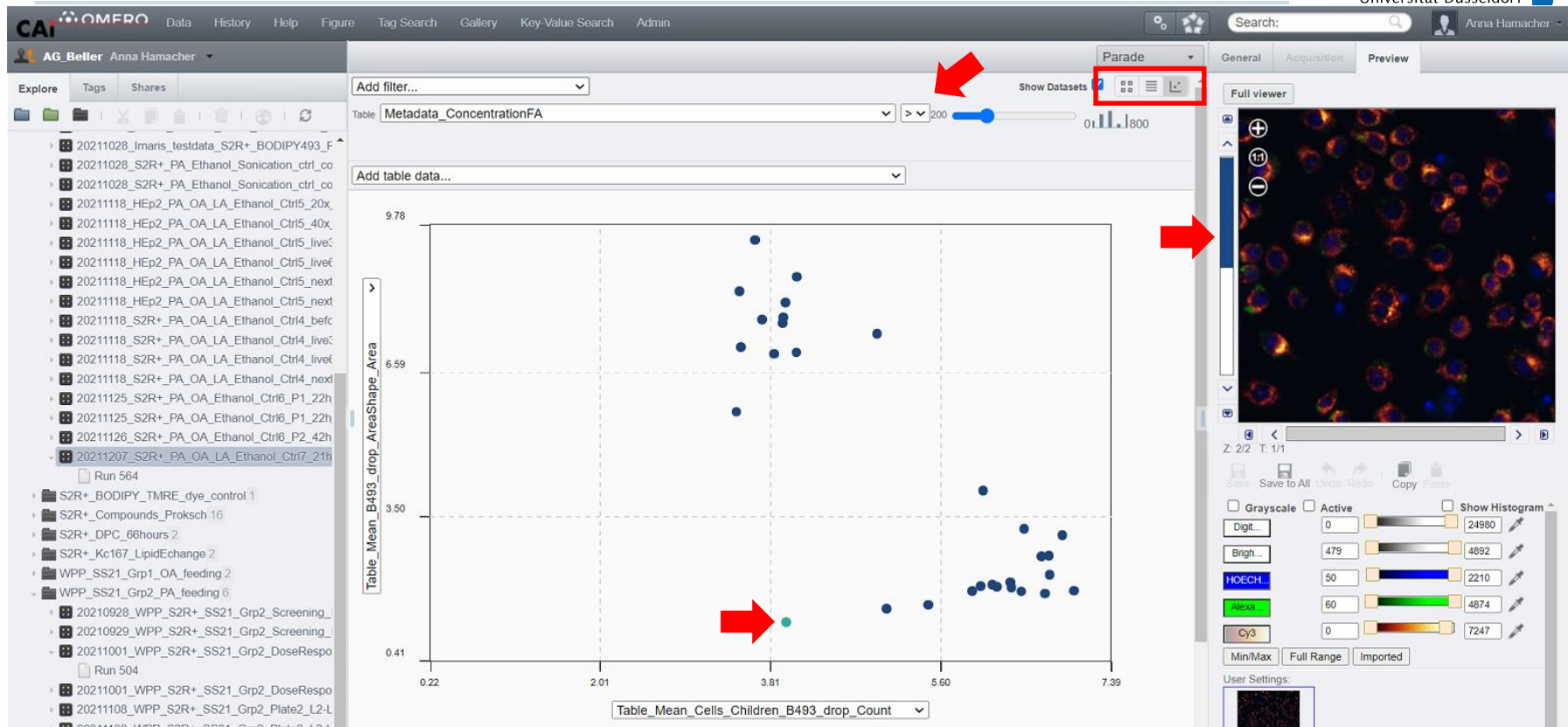
20210928\_WPP\_S2R+\_SS21\_Grp2\_Screening\_1

20210929\_WPP\_S2R+\_SS21\_Grp2\_Screening\_1

20211001\_WPP\_S2R+\_SS21\_Grp2\_DoseRespo

Run 504

# Plotting in OMERO.parade



# Exploring Plate Data

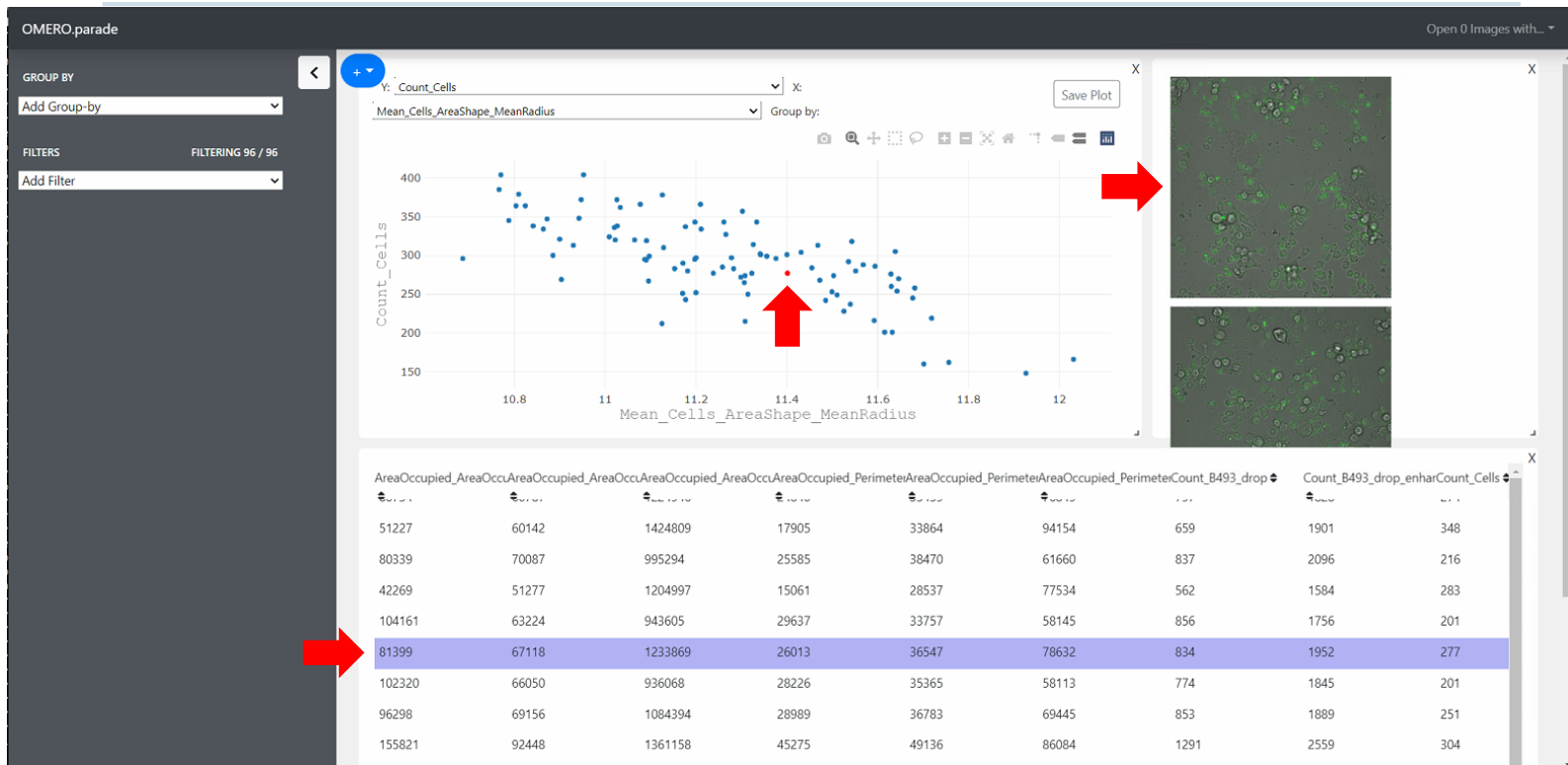
There are some limitations in OMERO.parade, so Will Moore (OME) has developed a prototype:

## OMERO.parade-crossfilter

- Screen exploration, not only single plates!
- Individual grouping
- Box plots
- Data exploration dashboard
- React webapp



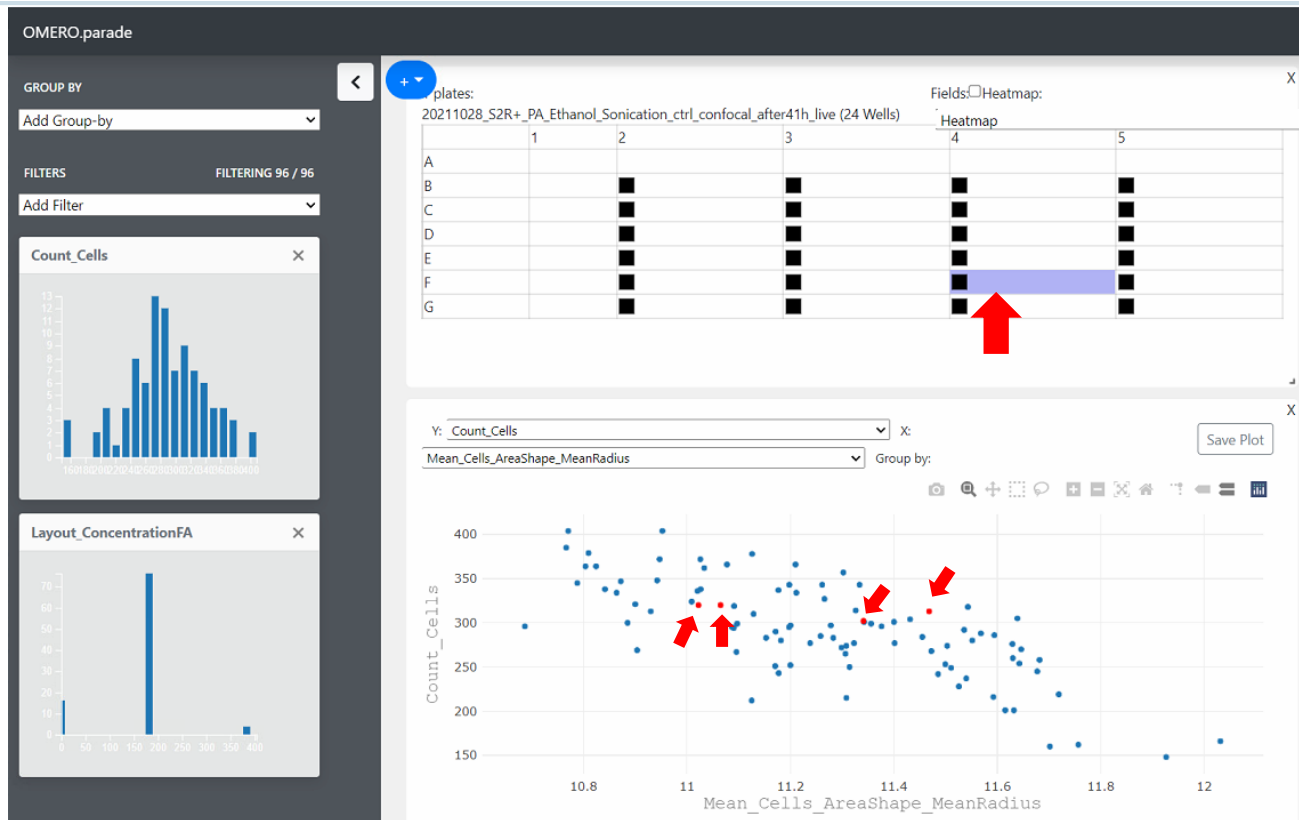
# OMERO.parade-crossfilter



■ Similar to parade

- Filter
- Scatter plot
- Table view
- Image preview

but interactive Dashboard!

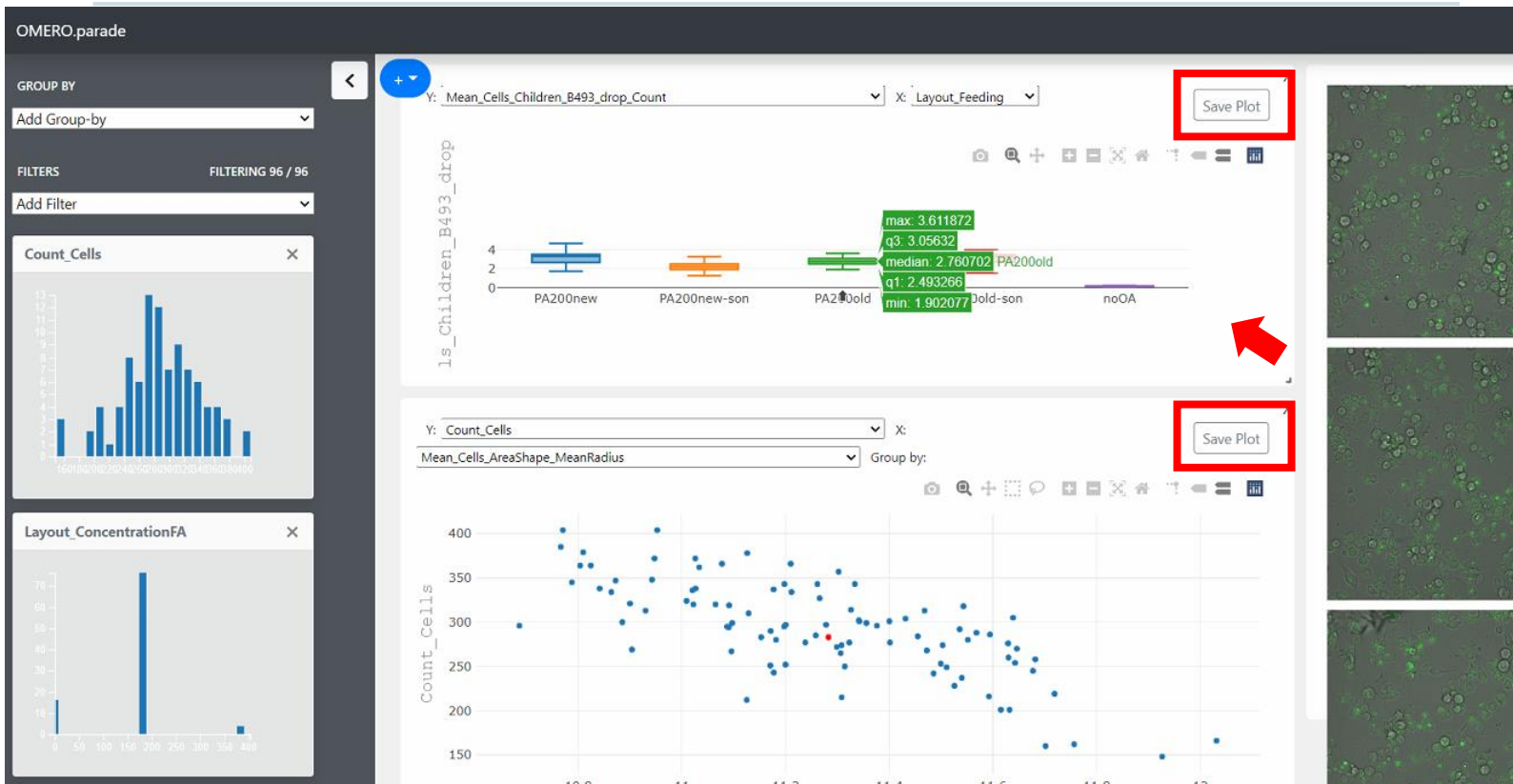


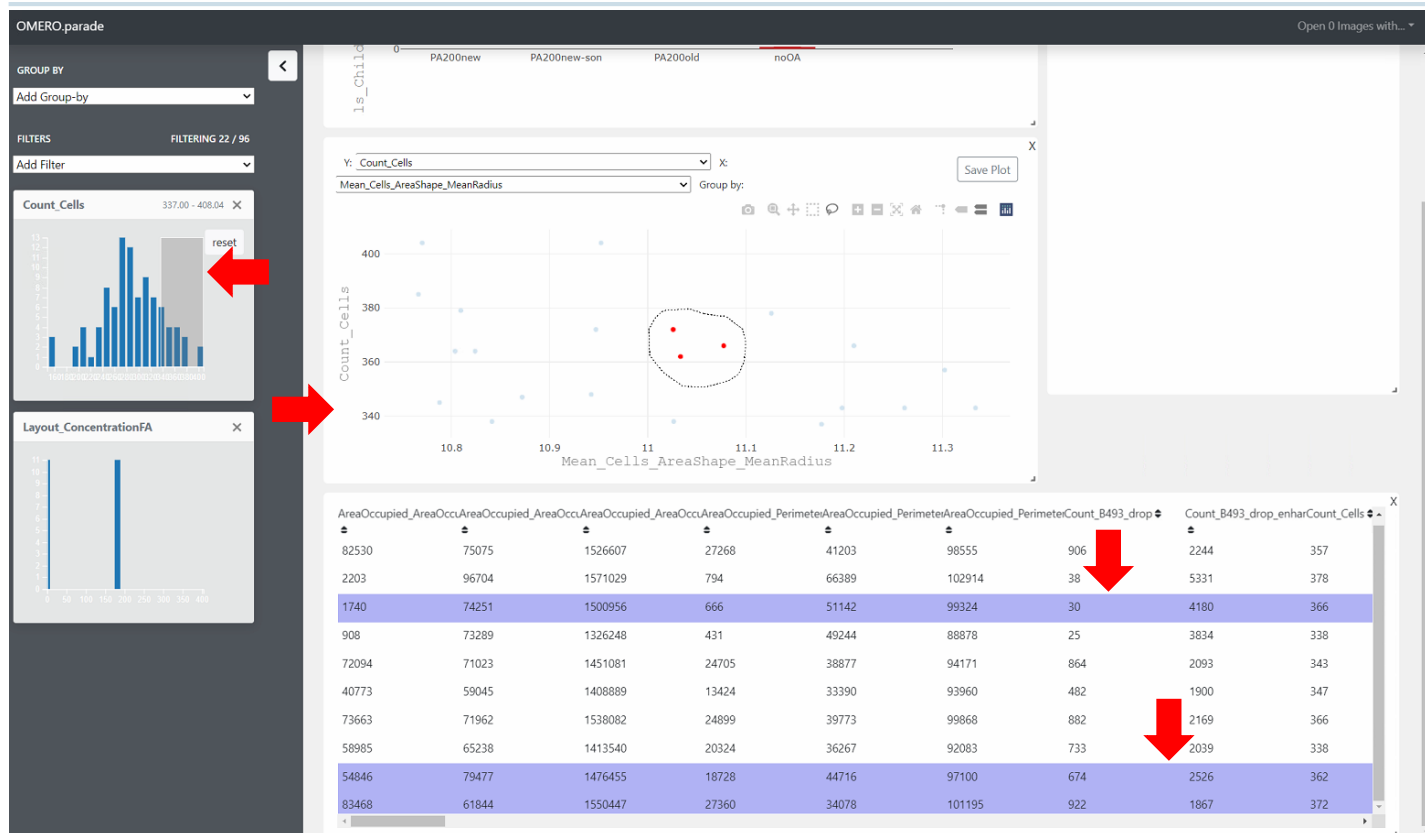
## New features

- Grouping
- Interactive heatmap
- Interactive charts (plotly)

## New features

- Box plots
- Save plots to OMERO





## New features

- Interactive filtering
- Interactive selection
- Everything is connected!

## ■ **Hardware** related:

- Long uploads, long processing
- **Performance** with many concurrent connections on the same plate (e.g. student courses)
- Data gets duplicated when uploading to OMERO (native PerkinElmer file format can't be imported in-place)

## ■ **Workflow and software** related:

- Hard-copy/duplicate works only on command line → integration into server-side-scripts needed
- **Metadata import** via `populate metadata` is too complex/error-prone → integration into server-side-scripts for more usability and user acceptance
- OMERO.parade-crossfilter has many nice features, but is not ready for production yet



Thanks for your attention!

