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OME Users' Meeting
June 18, 2012

JCB Data Viewer

# Bringing New Dimensions to Published Image Data

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Executive Editor, *The Journal of Cell Biology*
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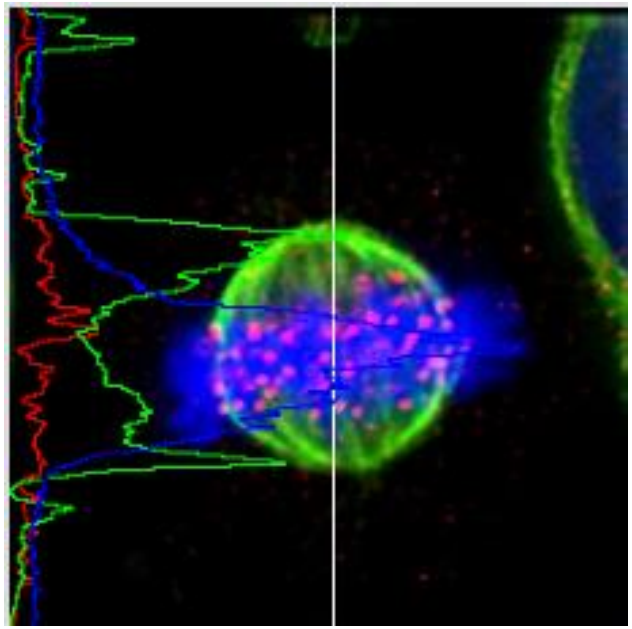
# Why does a publisher care about OME/OMERO?

**Our goal as a publisher:**

- To ensure the integrity of the scientific record.
- To ensure that what our readers see is what the authors saw.



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# Why does a publisher care about OME/OMERO?

**How do we ensure that what our readers see is what the authors saw?**

- by promoting access to original image data
- by promoting sharing of original image data
- by promoting archiving of original image data

OME/OMERO and the JCB Data Viewer

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# JCB Data Viewer

- An OMERO-based, browser-based application for archiving, viewing, and sharing original, raw image files associated with *JCB* papers.
- Enables presentation and archiving of the original data as acquired by the user, pre-manipulation.
- Enables hosting and sharing of multidimensional, multichannel files from various types of imaging systems.
- Allows users (editors, reviewers, readers) to perform simple analyses of the data within the application and download the data in the open OME-TIFF format for more detailed analysis with their software of choice.
- Enables multidimensional publishing – far beyond what is possible with standard PDFs and html.

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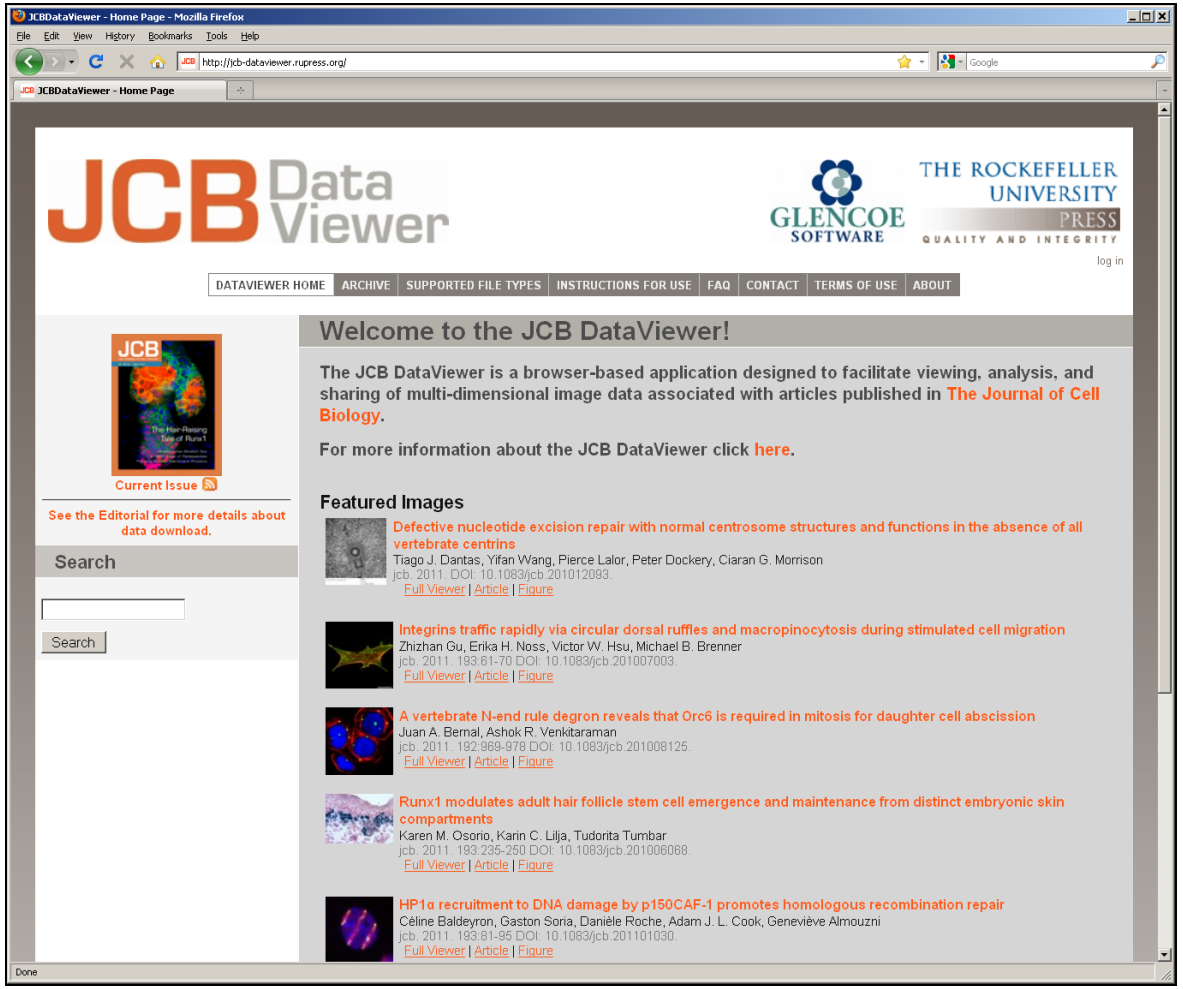


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# File Types Supported (using Bio-Formats)

Amira Mesh (.am, .hx, etc.)

Olympus Slidebook (.sld)

DeltaVision (.dv, .r3d, etc.)

Prairie Technologies TIFF (.tif,.xml,.cfg)

PerkinElmer Opera Flex (.flex)

MINC MRI (.mnc)

Gatan digital micrograph (.dm3)

IPLab (.ipl)

Alicona 3D (.al3d)

Metamorph STK (.stk, .nd)

Zeiss Laser-Scanning Micro. (.lsm,mdb)

Cellomics (.c01)

InCell 1000 (.xdce, .tif)

Zeiss AxioVision ZVI (.zvi)

Bitplane Imaris (.ims)

Maia Scientific (.tif)

IVision (.ipm)

PerkinElmer (.ano, .cfg, .tim, etc.)

Nikon ND2 (.nd2)

Visitech XYS (.xys, .html)

Hamamatsu Aquacosmos NAF (.naf)

Bio-Rad PIC (.pic, .xml, .raw)

TillPhotonics TillVision (.vws)

DICOM (.dcm, .dic, etc.)

Openlab LIFF (.liff)

Leica (.lei, .tif, .tiff)

Nikon NEF (.nef, .tif, .tiff)

FITS (.fits)

Li-Cor L2D (.l2d, .scn, .tif)

ImagePro Workspace (.ipw)

Olympus APL (.apl, .tnb, .mpb)

Aperio SVS TIFF (.svs)

Improvision TIFF (.tif)

And many more – more than 75 PFFs and growing.

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# 1. Single Image Analysis

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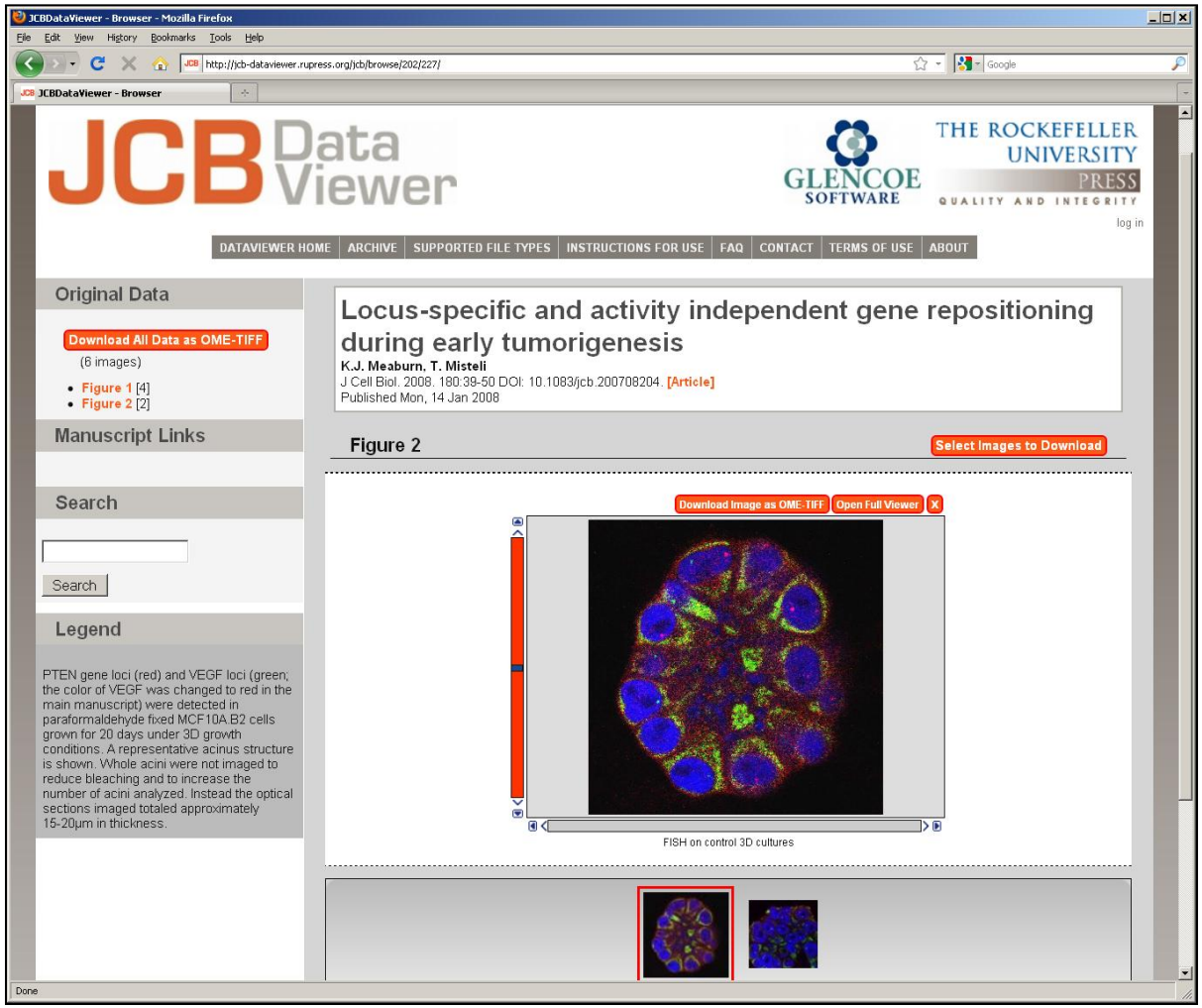
## The Mini Viewer



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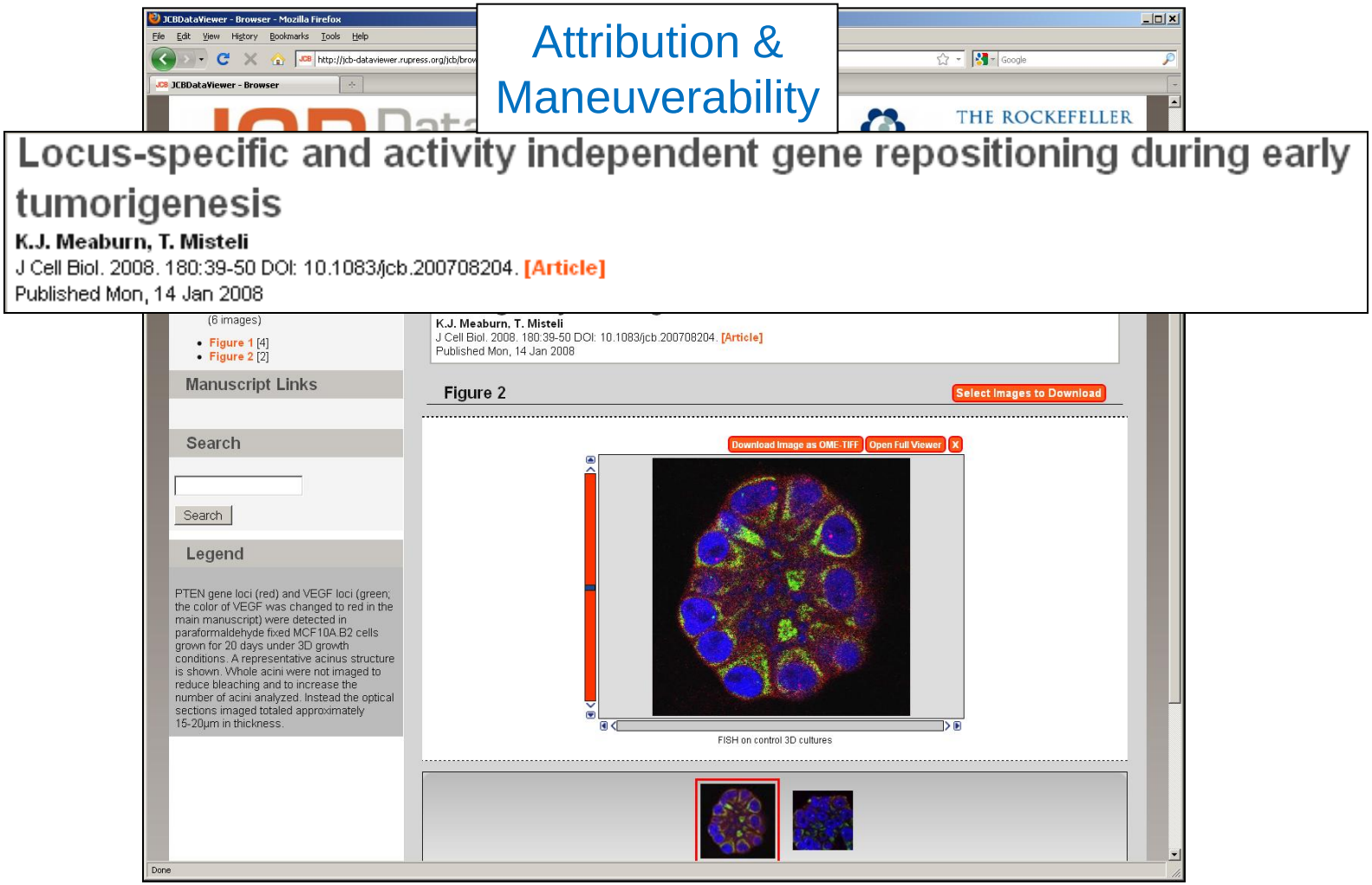
# The Mini Viewer

Attribution & Maneuverability



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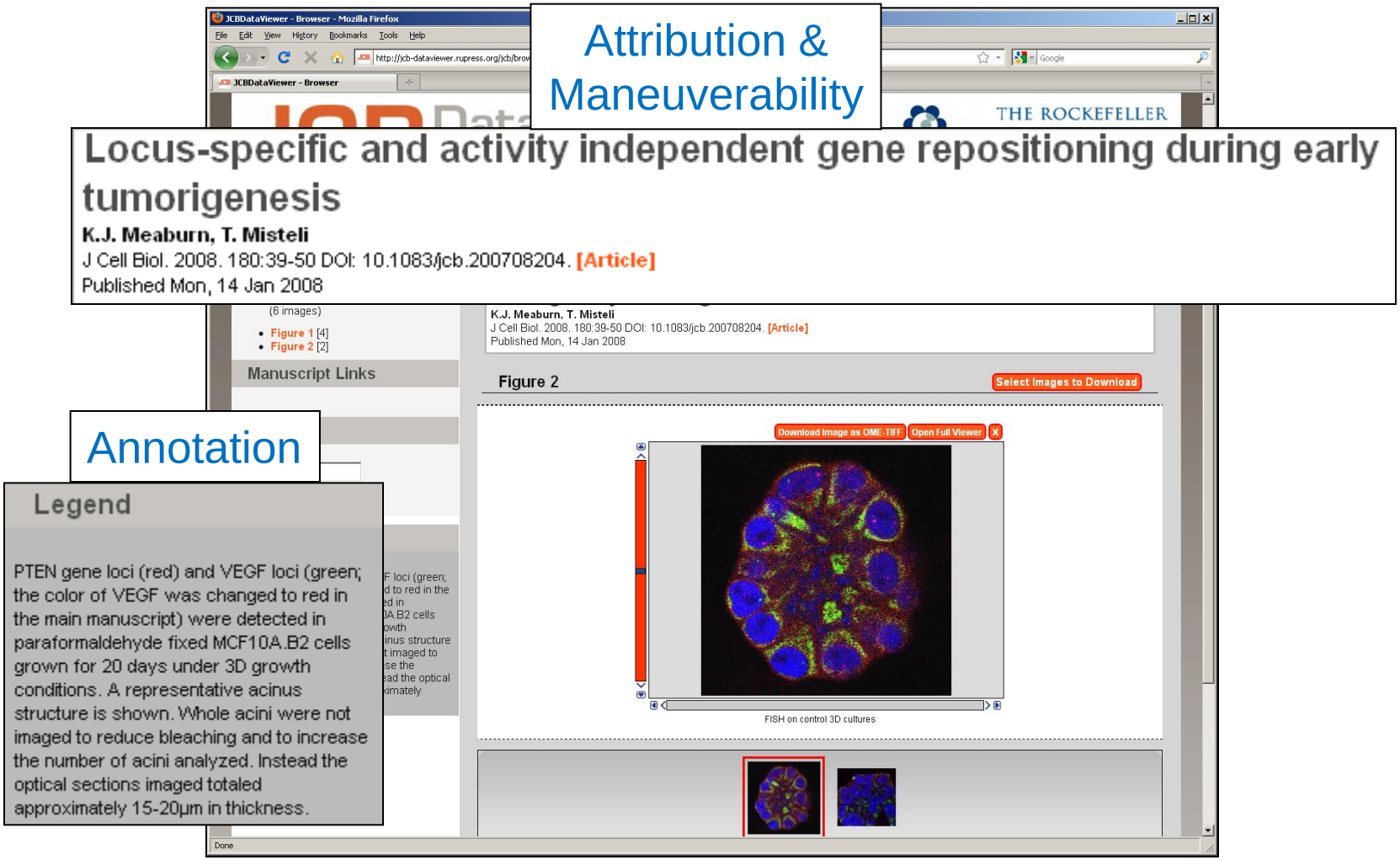
# The Mini Viewer

Attribution & Maneuverability

Annotation



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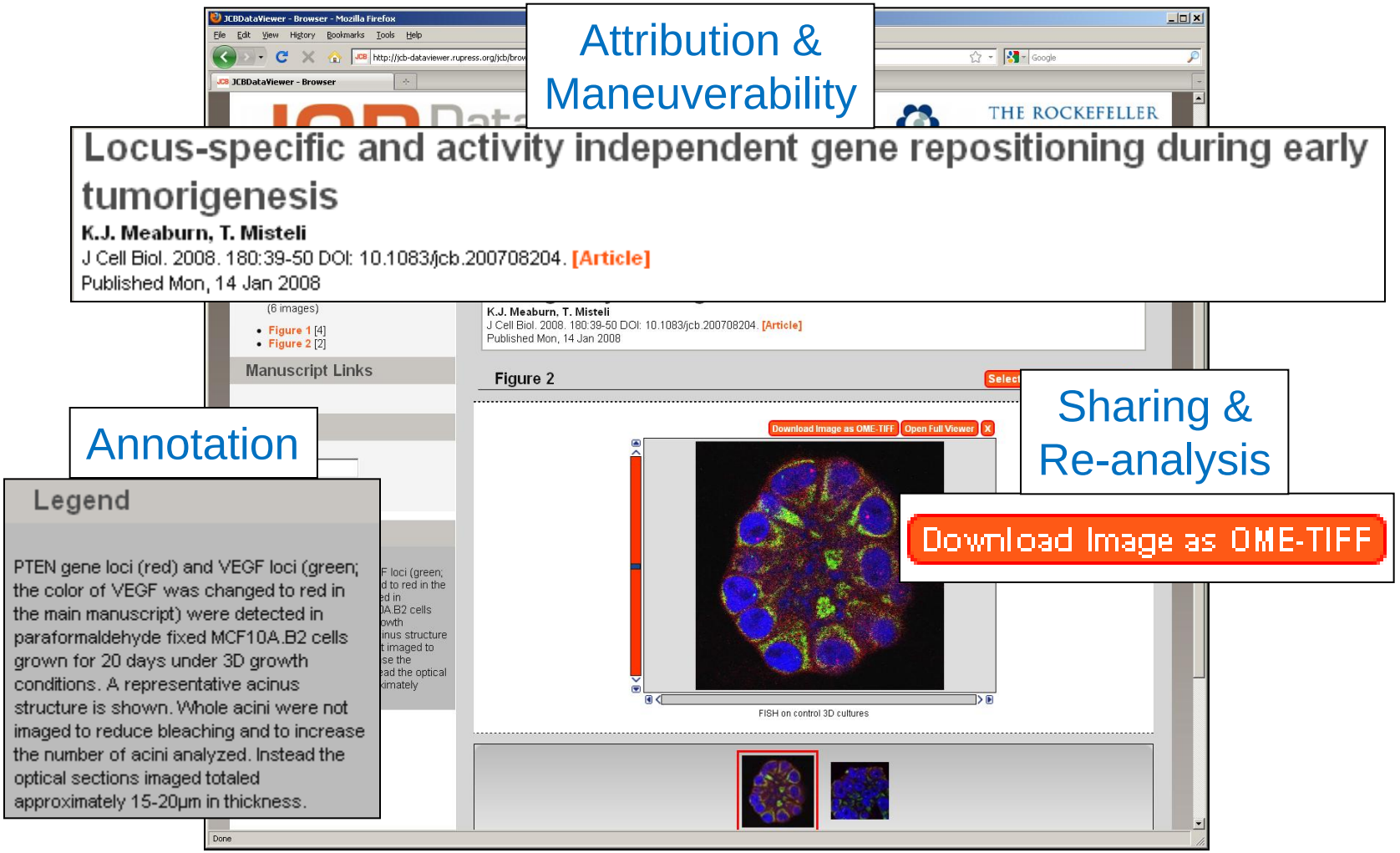

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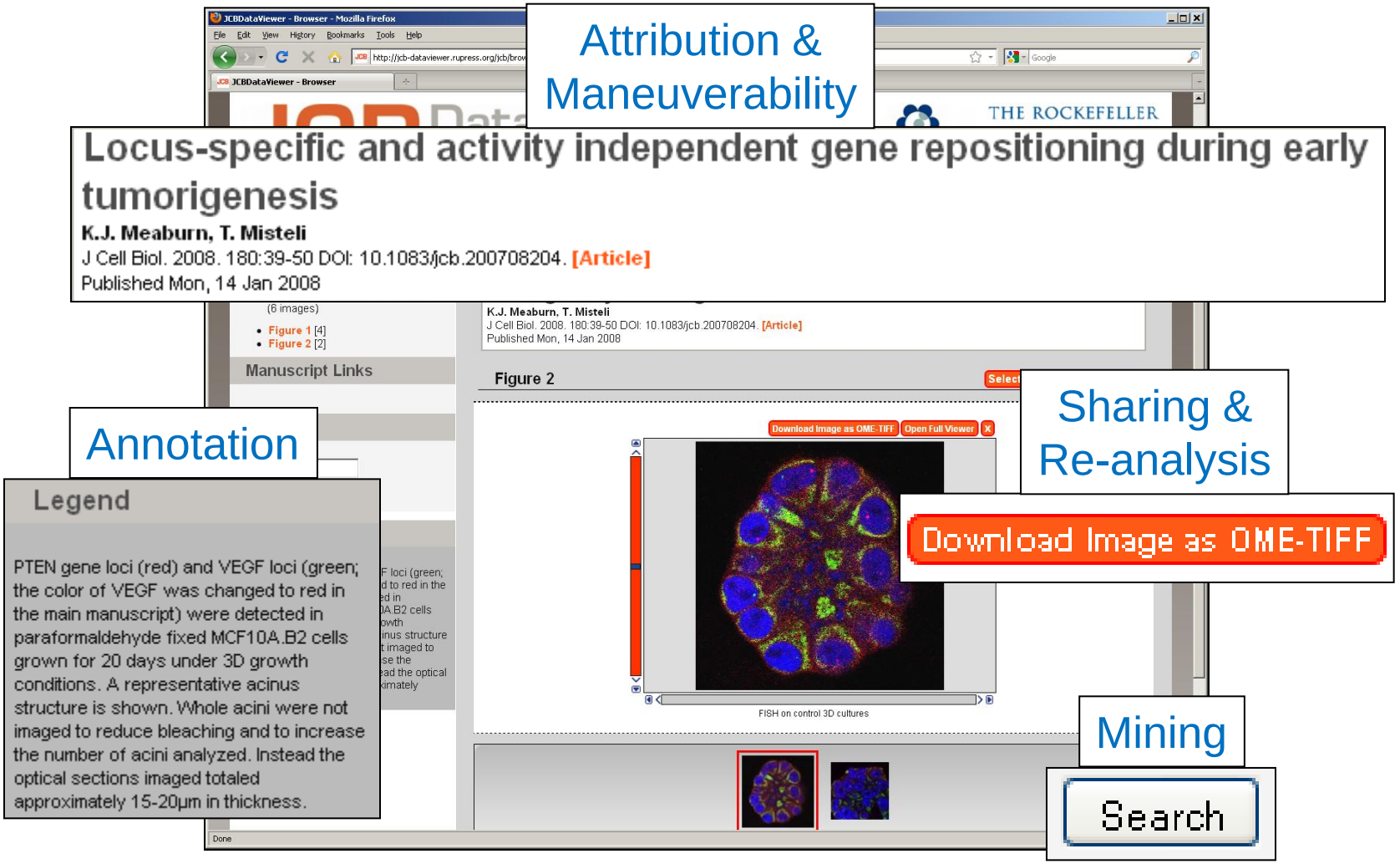
## The Mini Viewer



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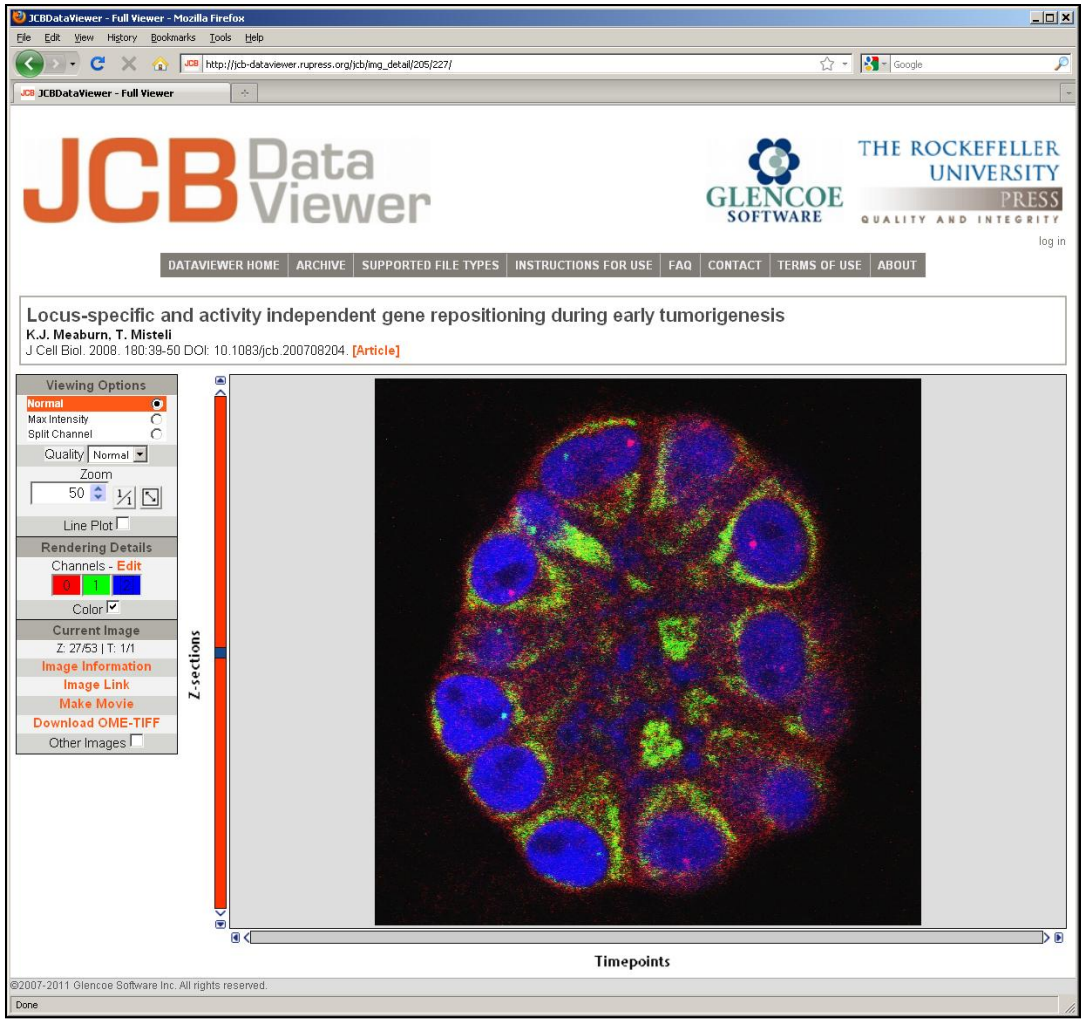

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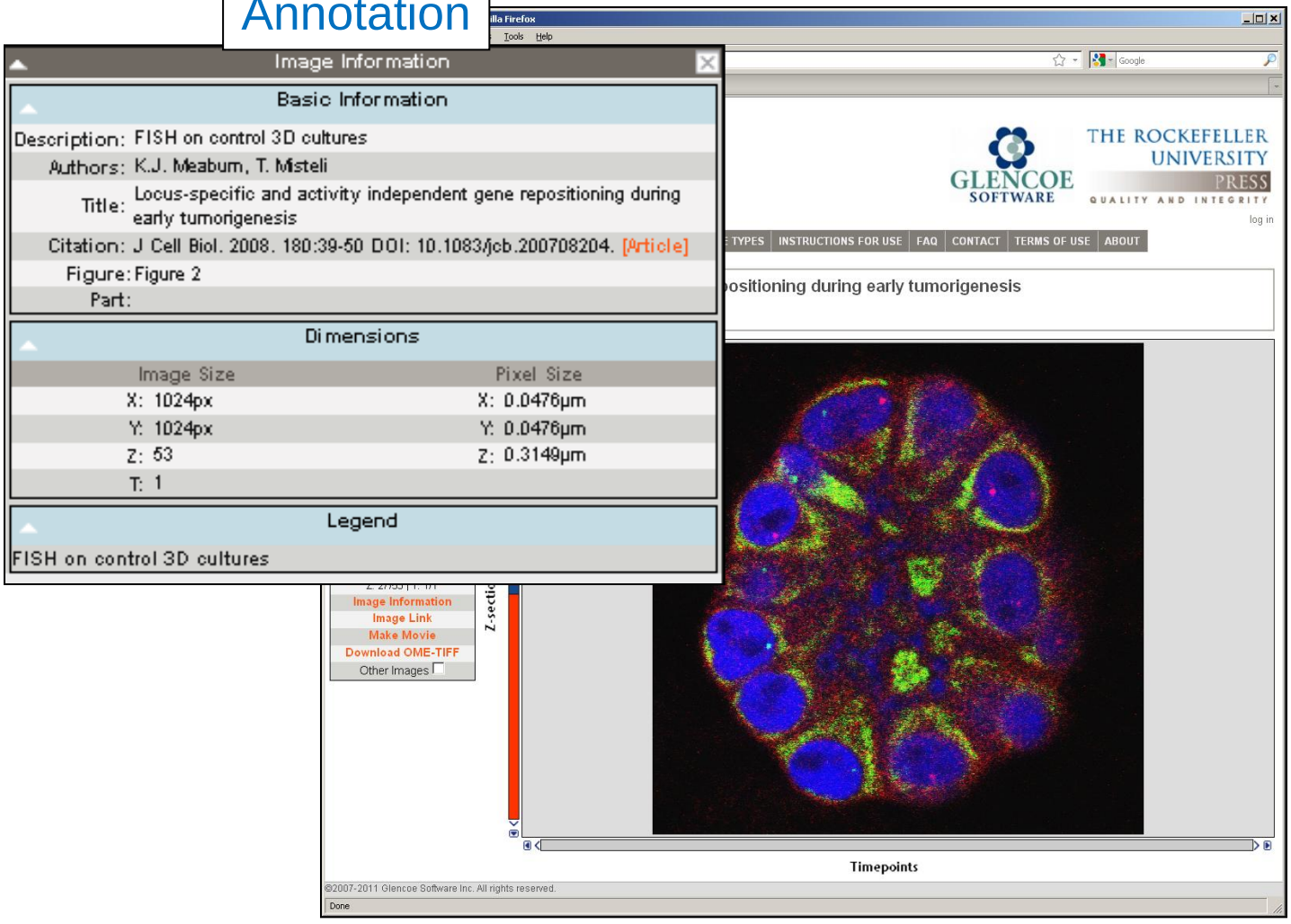
Annotation



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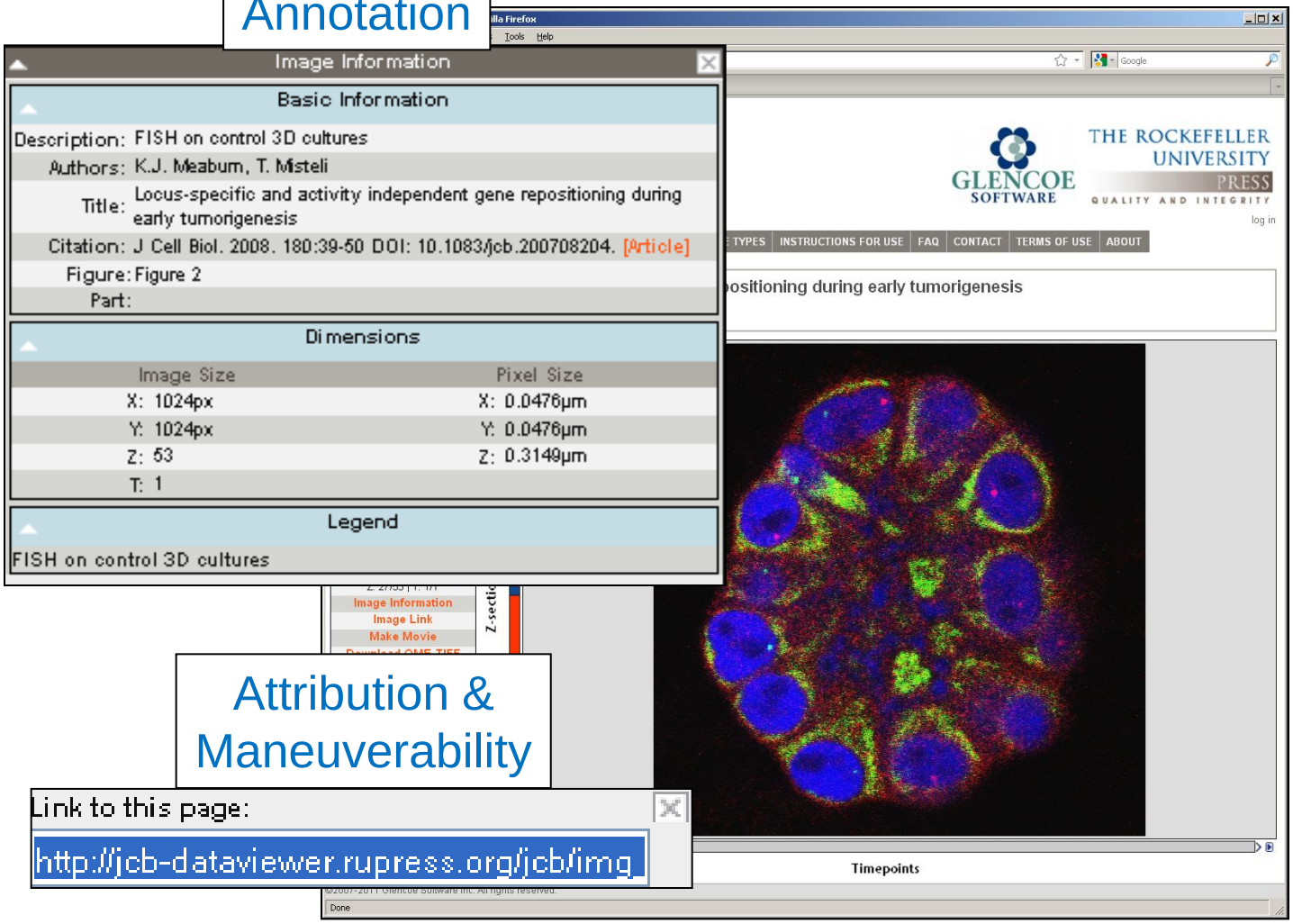
Annotation

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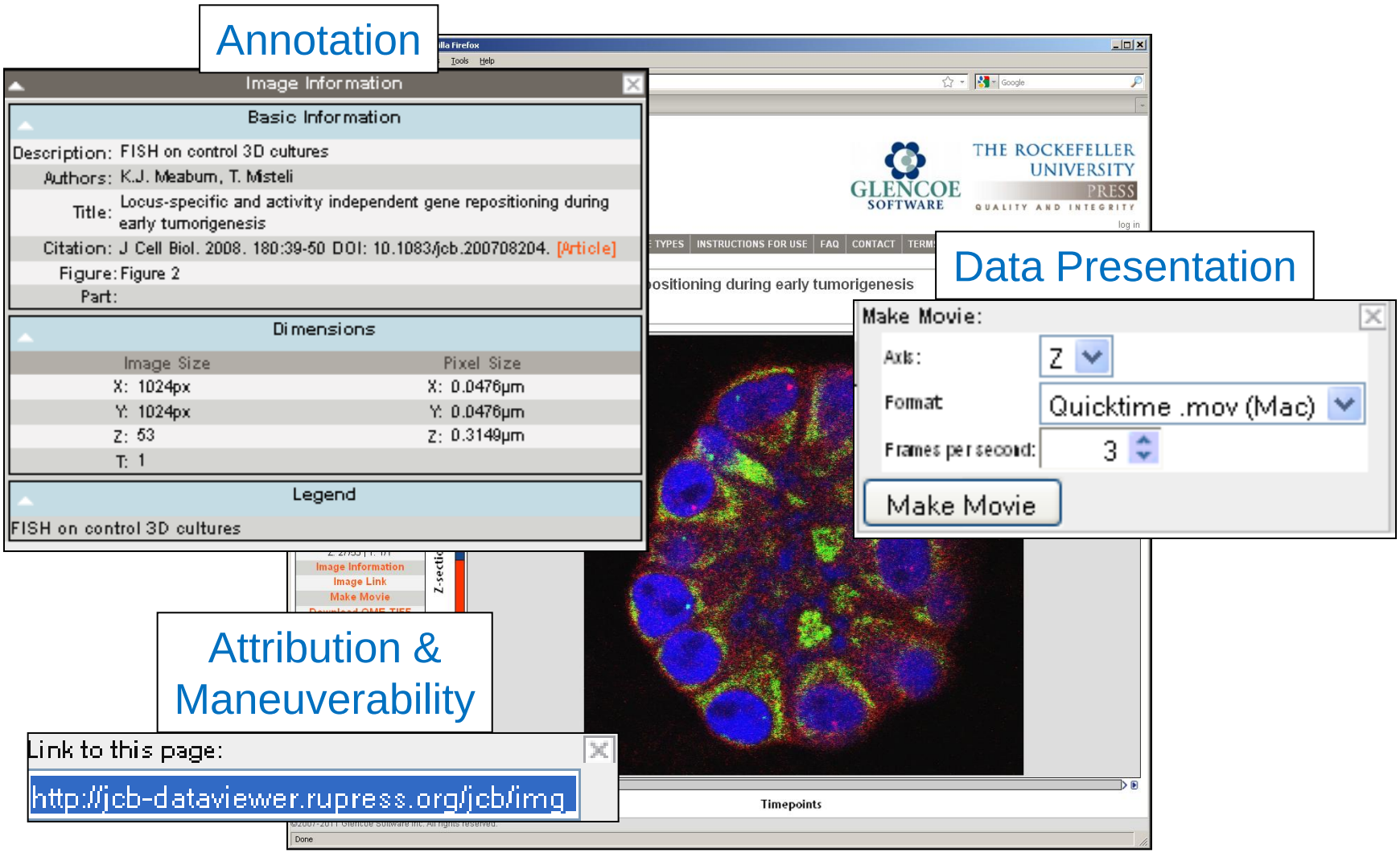

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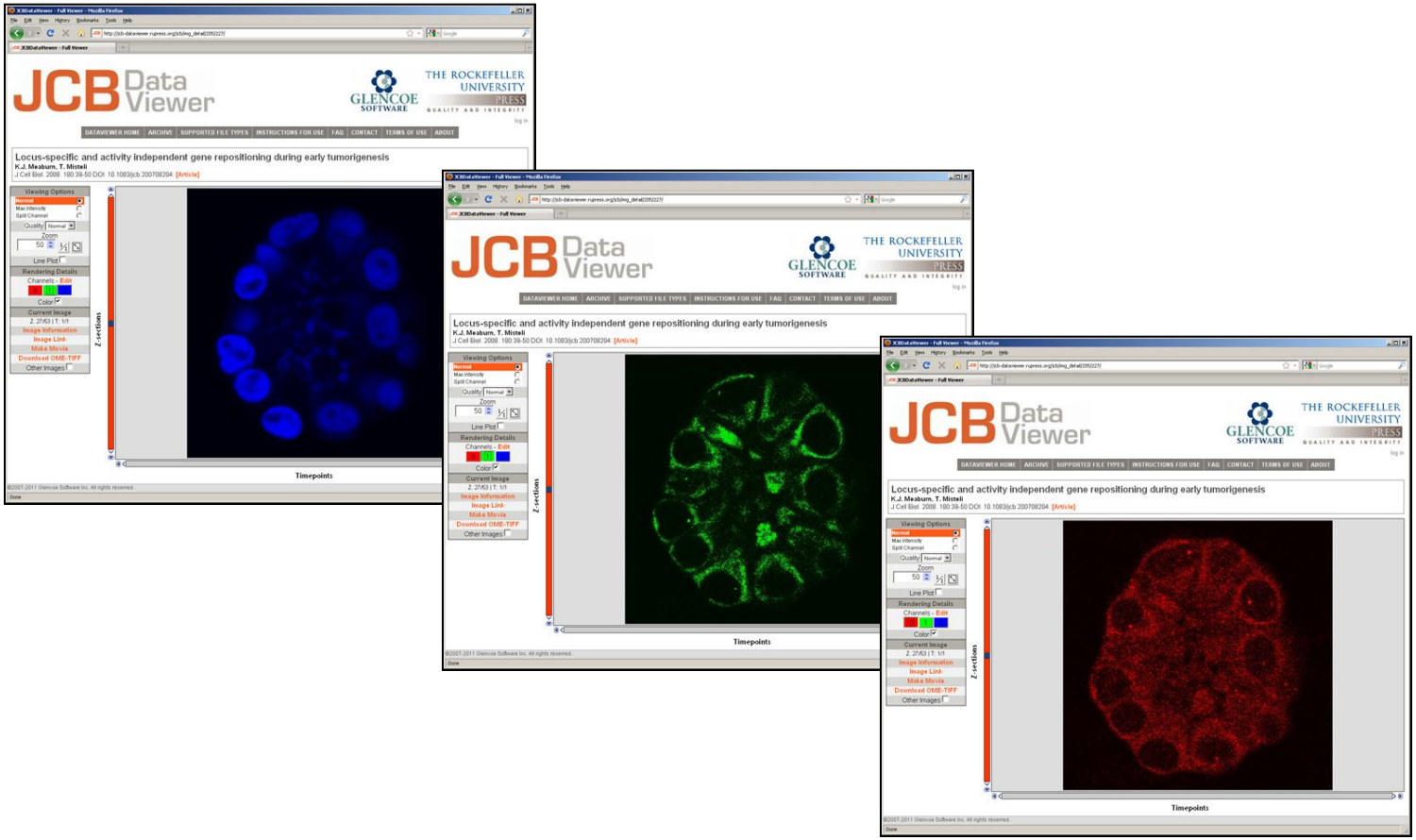
# Split-Channel View



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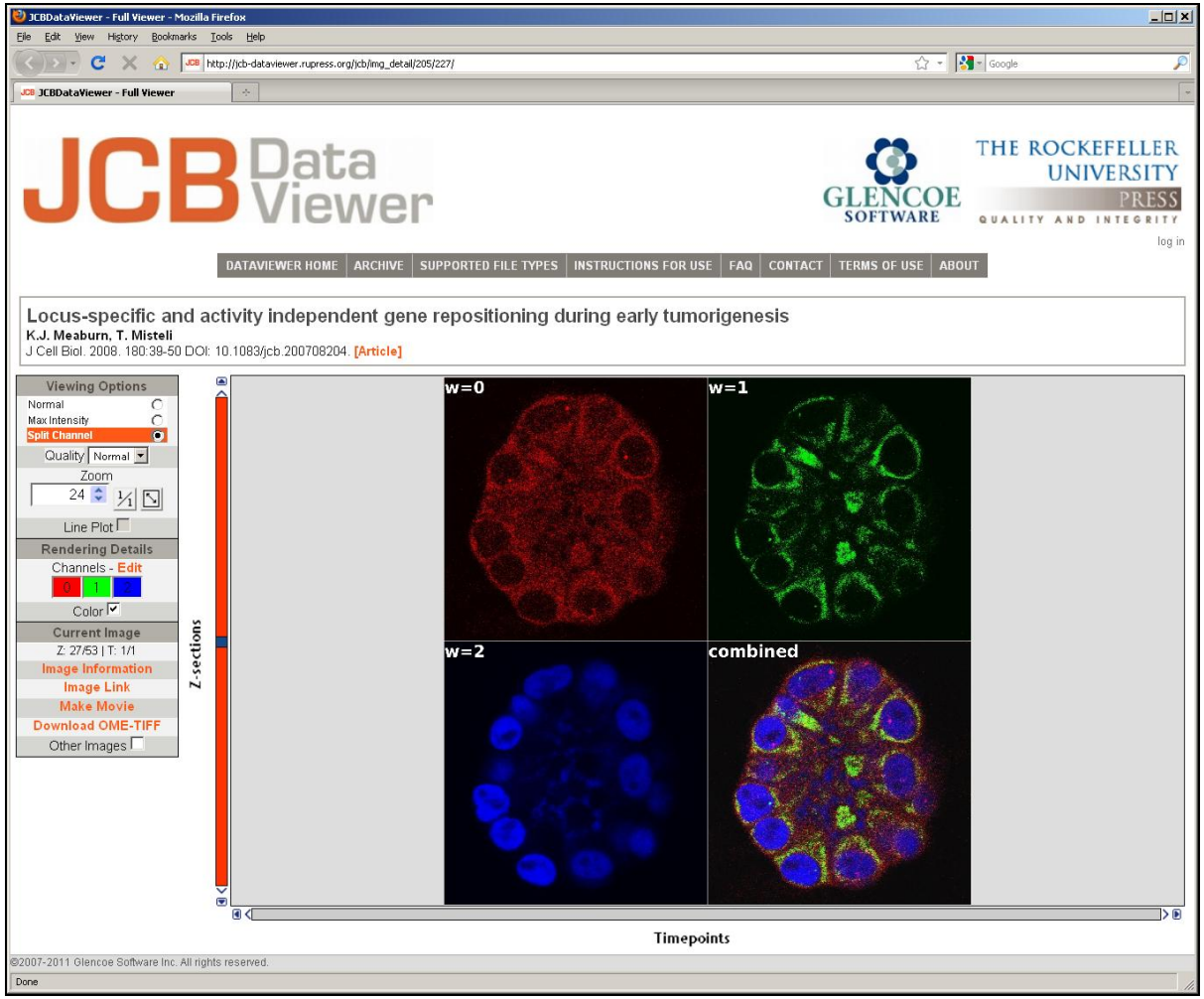
# Split-Channel View



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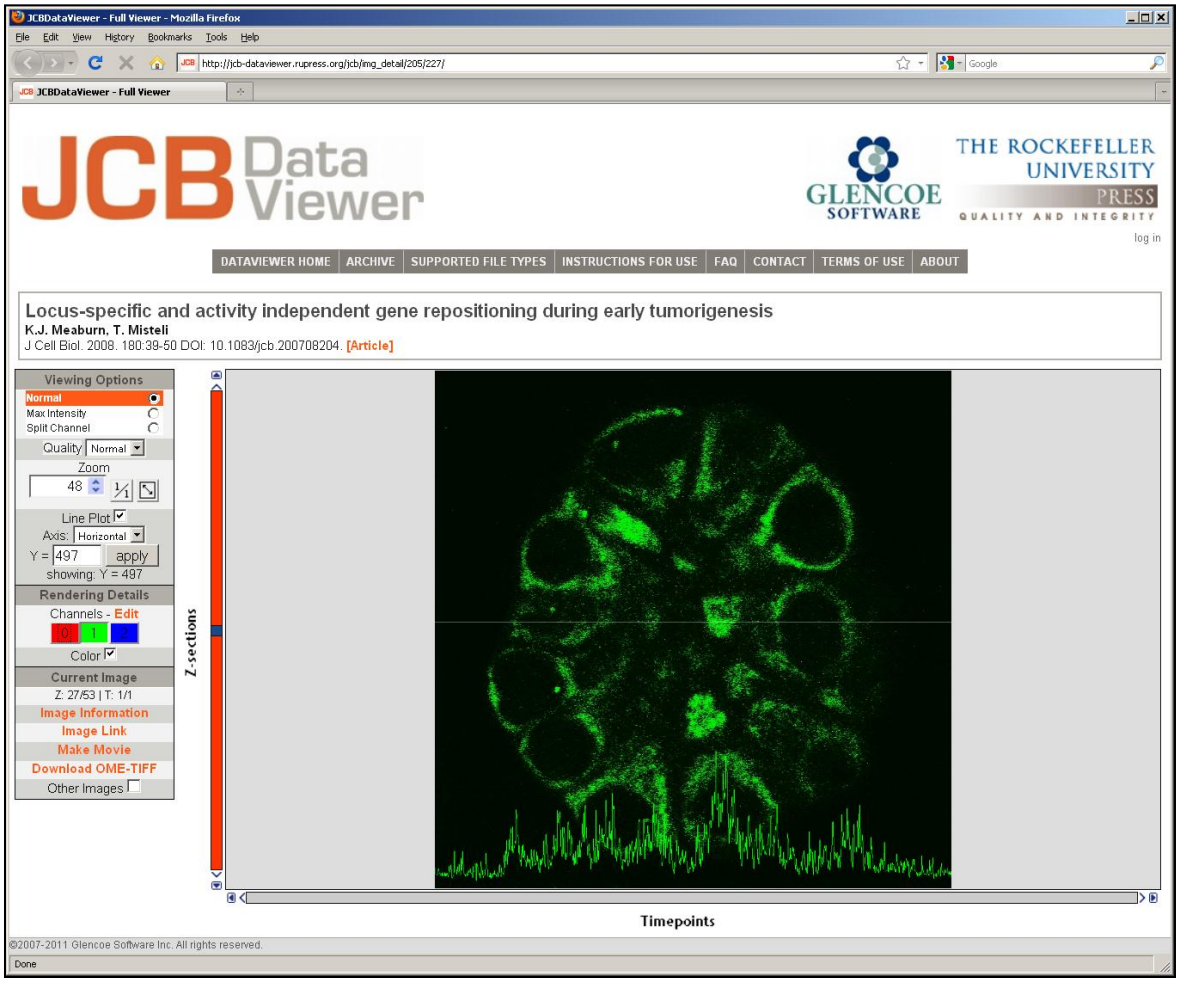
# Line plots



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# 2. High-Content Screen Analysis

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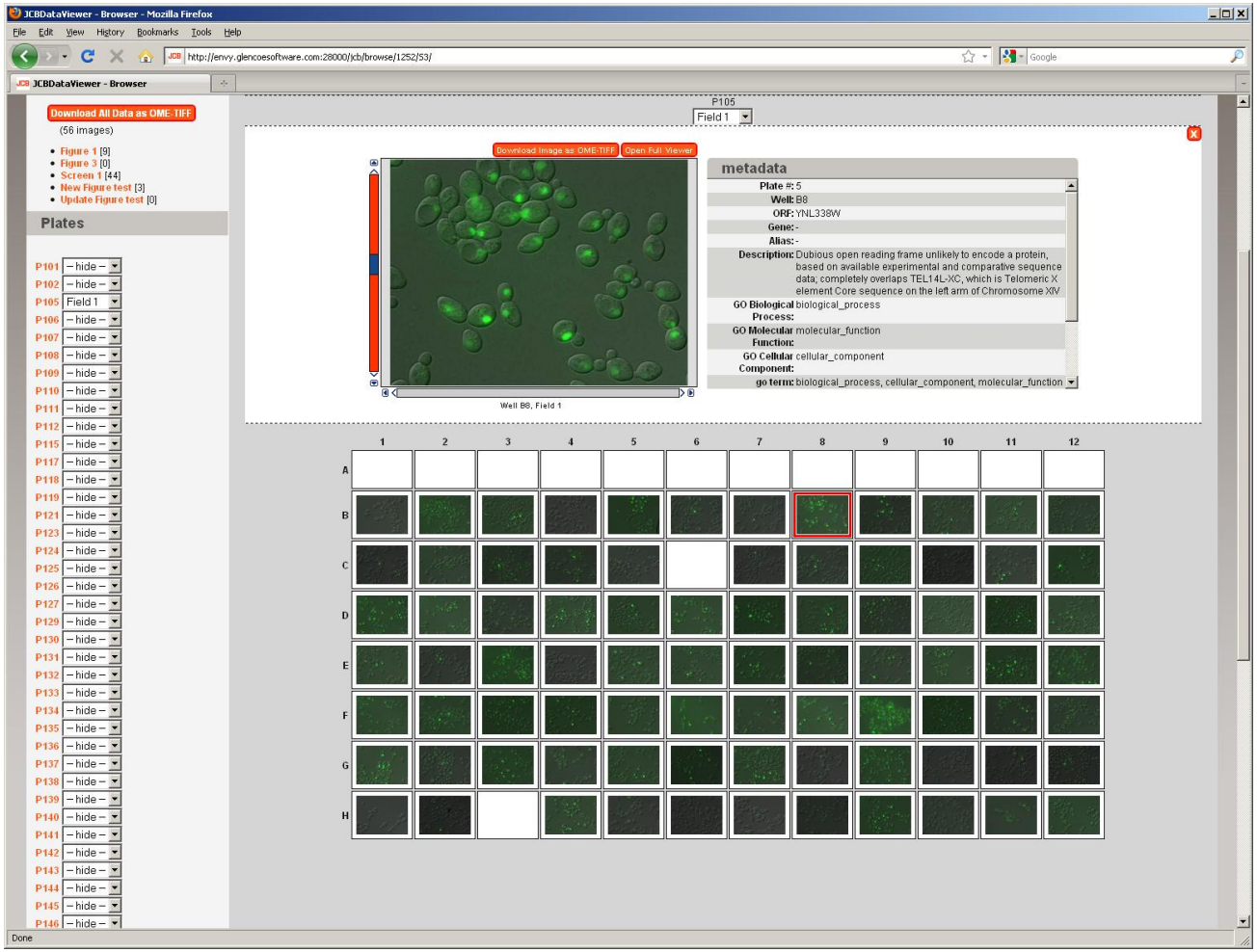
# High-Content Screen Data



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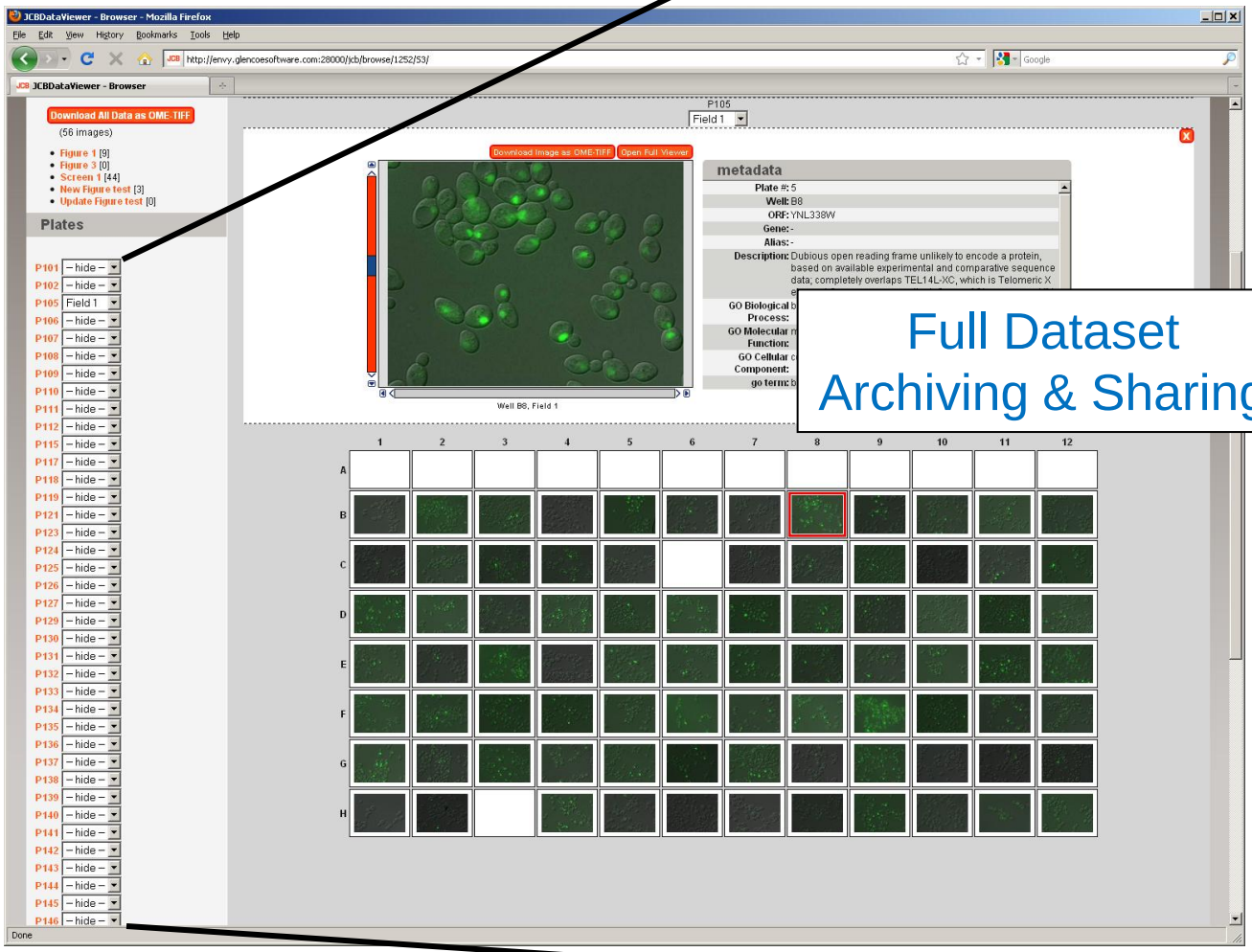
# High-Content Screen Data




Full Dataset
Archiving & Sharing

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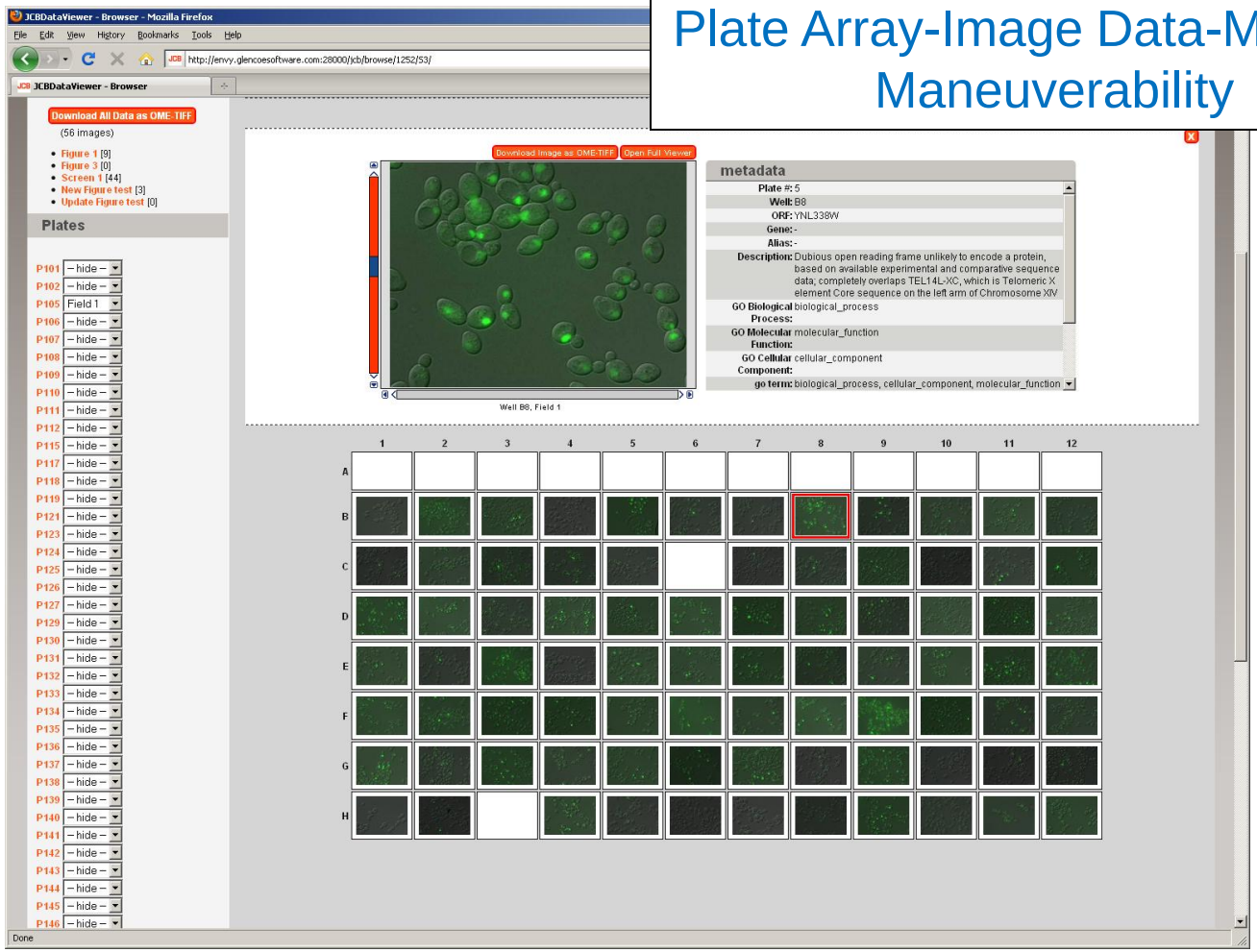
# High-Content Screen Data

Plate Array-Image Data-Metadata Maneuverability



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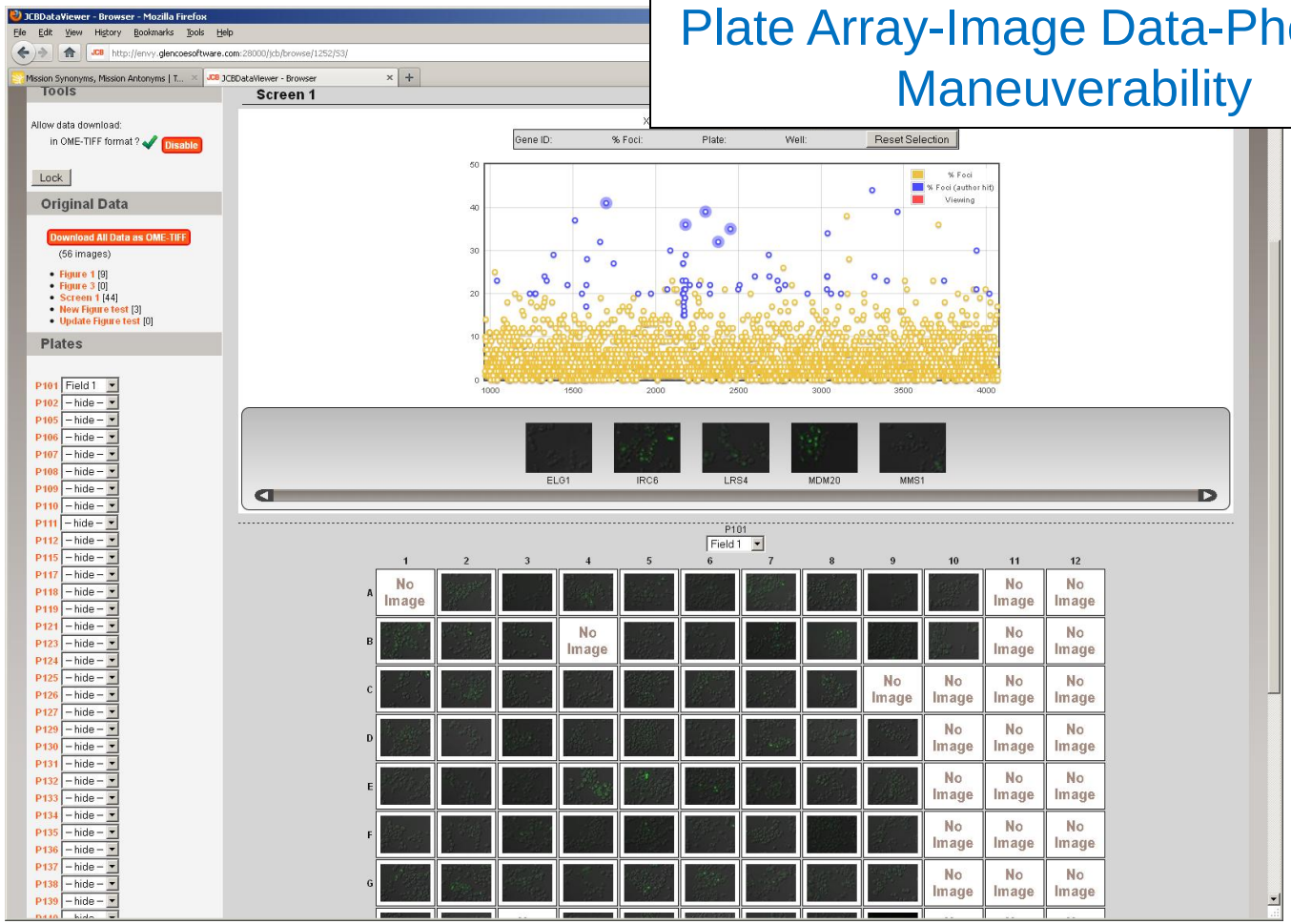
# High-Content Screen Data

Plate Array-Image Data-Phenotype Maneuverability



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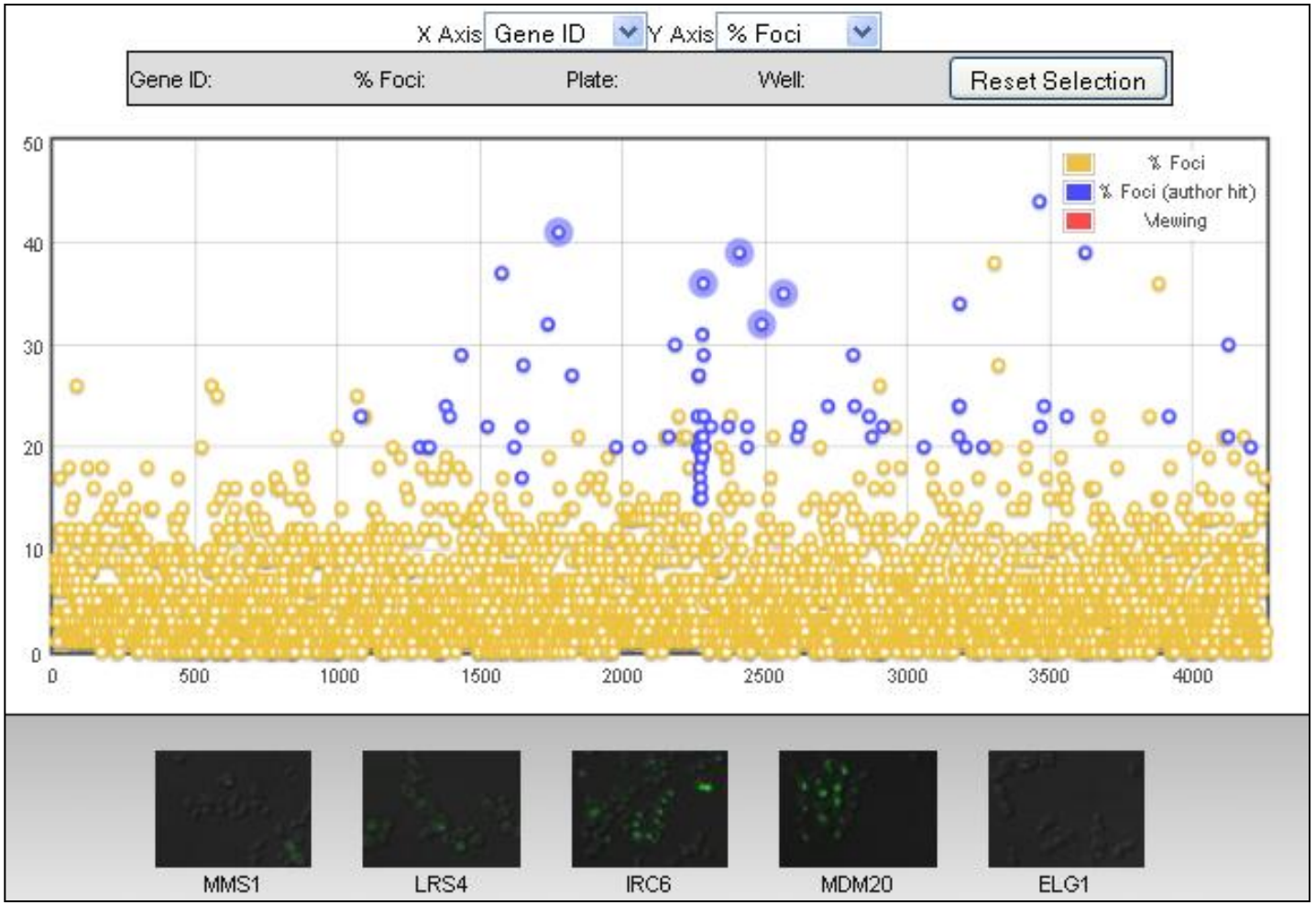
# High-Content Screen Data



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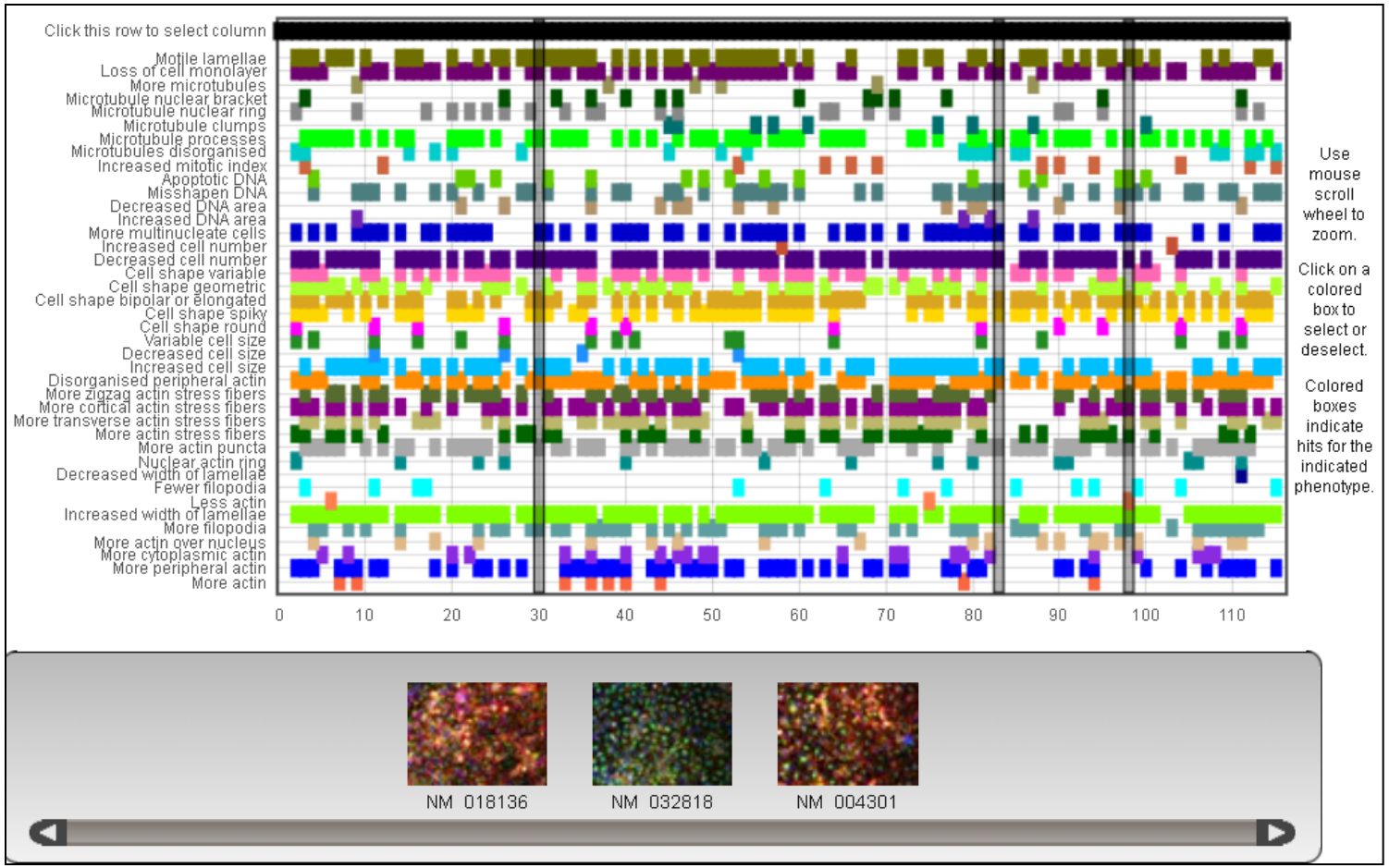
# High-Content Screen Data



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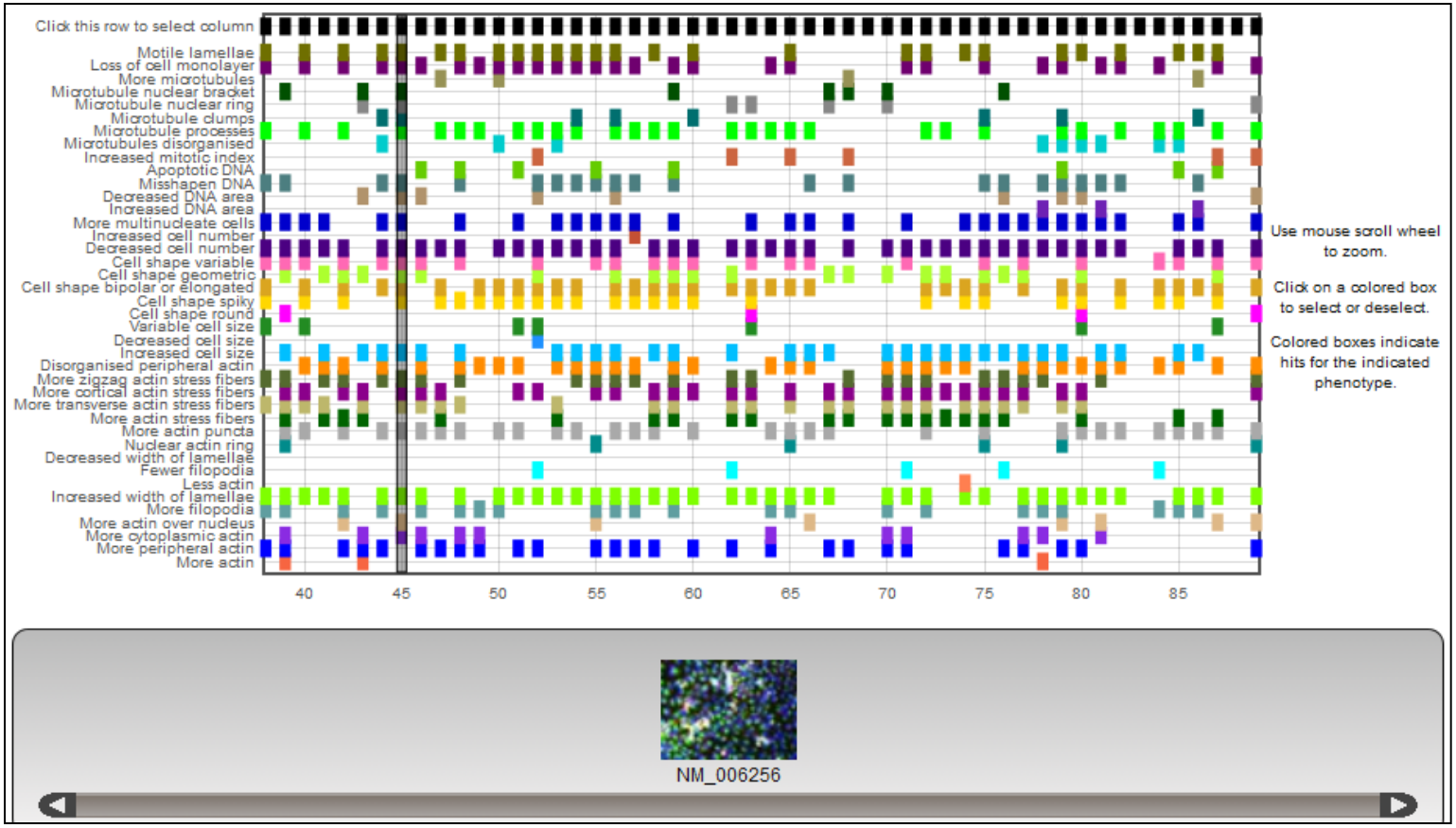
## High-Content Screen Data



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# 3. Tiled Image Analysis

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# Coming soon: BIG images

**Ultra-large, high-resolution, tiled images:**

- 921600 pixels x 380928 pixels
- 281 gigapixels total
- 16 GB
- 1.6 nm resolution component images

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# Ultra-large, high-resolution, tiled images

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# Multidimensional publishing

difference in the amount of Aurora B in unaligned and apparently aligned chromosomes (Fig. 5 A). We detected no change in chromosome staining with anti–phosphohistone H3 (Fig. 5 A) or anti-phospho–CENP-A (not depicted) after Bod1 depletion. Because both are markers of Aurora B activity (Zeitlin et al., 2001), these results suggest that Aurora B kinase activity was not dramatically impaired by the loss of Bod1. To further assay the function of Aurora B, we determined the localization of MCAK, which localizes to the inner centromere in its phosphorylated form but concentrates at kinetochores in its dephosphorylated state (Andrews et al., 2004). At unaligned sister kinetochores or in kinetochore pairs not yet fully under tension, MCAK is predominantly located at the inner centromere (Fig. 5 B; Andrews et al., 2004). In Bod1$^{siRNA}$ cells, we observed that although total MCAK present at unaligned centromeres was similar to control cells (Fig. 5 C), its precise localization was abnormal, forming multiple foci stretching out to one or both sister kinetochores.

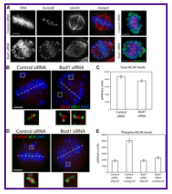

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Figure 5. **MCAK is not efficiently phosphorylated in Bod1$^{siRNA}$ cells.** (A) Aurora B is not delocalized in Bod1-depleted cells. Phospho-Ser10-histone H3 staining in control and Bod1 siRNA cells indicating Aurora B activity. (B–E) Cells were transfected with control or Bod1 siRNA. After 72 h, cells were treated with monastrol for 3 h and released into media containing MG132 for 1 h before fixing. (B and C) Cells were stained for total MCAK population, and levels at kinetochores were quantified. Boxed areas are magnified below the main images. (D and E) Cells were stained for phospho-Ser92-MCAK, and levels at aligned and unaligned kinetochores were quantified. Dashed lines indicate orientation of the metaphase plate. Error bars represent SD. Bars, 5 μm.

Because MCAK localization to centromeres and kinetochores depends on the state of MCAK phosphorylation, we examined the levels of phosphorylated MCAK using an anti–phospho-Ser92 MCAK antibody (Andrews et al., 2004). Phosphorylation of MCAK was substantially reduced at the inner centromere of unaligned chromosomes in Bod1$^{siRNA}$ cells compared with the control cells (Fig. 5, D and E). These results suggest that Bod1 depletion impairs the formation of bioriented attachments across sister kinetochores, possibly by impairing the correct localization of MCAK at centromeres and, thereby, preventing its phosphorylation and timely correction of syntelic attachments. We have not detected any effect of Bod1 on the in vitro phosphorylation of MCAK by Aurora B (unpublished data), so Bod1 may modulate MCAK phosphorylation by interacting with other proteins. Aurora B activity and kinetochore oscillations are necessary for syntelic correction (Lampson et al., 2004), and our data further suggest that syntelic correction may require MCAK phosphorylation. Whether there is any subtle perturbation in kinetochore oscillations in Bod1-depleted cells is not yet known and will require much higher resolution live cell imaging.

In summary, by using a cell cycle–dependent analysis of the *Xenopus* chromatin proteome, we have identified a novel protein required for proper chromosome biorientation called Bod1. Bod1 is a member of the FAM44 protein family and is highly conserved throughout metazoans. Depletion of Bod1 in human cells causes severe biorientation defects, although kinetochores appear to

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# Multidimensional publishing

difference in the amount of Aurora B in unaligned and apparently aligned chromosomes (Fig. 5 A). We detected no change in chromosome staining with anti–phosphohistone H3 (Fig. 5 A) or anti-phospho–CENP-A (not depicted) after Bod1 depletion. Because both are markers of Aurora B activity (Zeitlin et al., 2001), these results suggest that Aurora B kinase activity was not dramatically impaired by the loss of Bod1. To further assay the function of Aurora B, we determined the localization of MCAK, which localizes to the inner centromere in its phosphorylated form but concentrates at kinetochores in its dephosphorylated state (Andrews et al., 2004). At unaligned sister kinetochores or in kinetochore pairs not yet fully under tension, MCAK is predominantly located at th
control cells (Fig. 5 C), its

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Because MCAK localizati
anti–phospho-Ser92 MCA
compared with the control
the correct localization of M
vitro phosphorylation of M
oscillations are necessary f
perturbation in kinetochore

In summary, by using a cell
is a member of the FAM4

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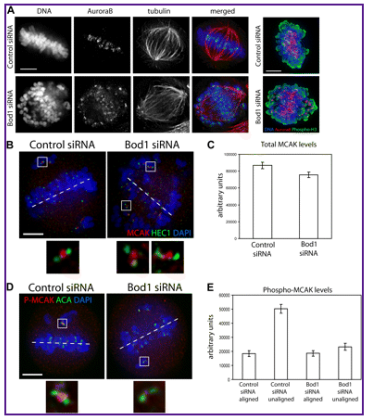


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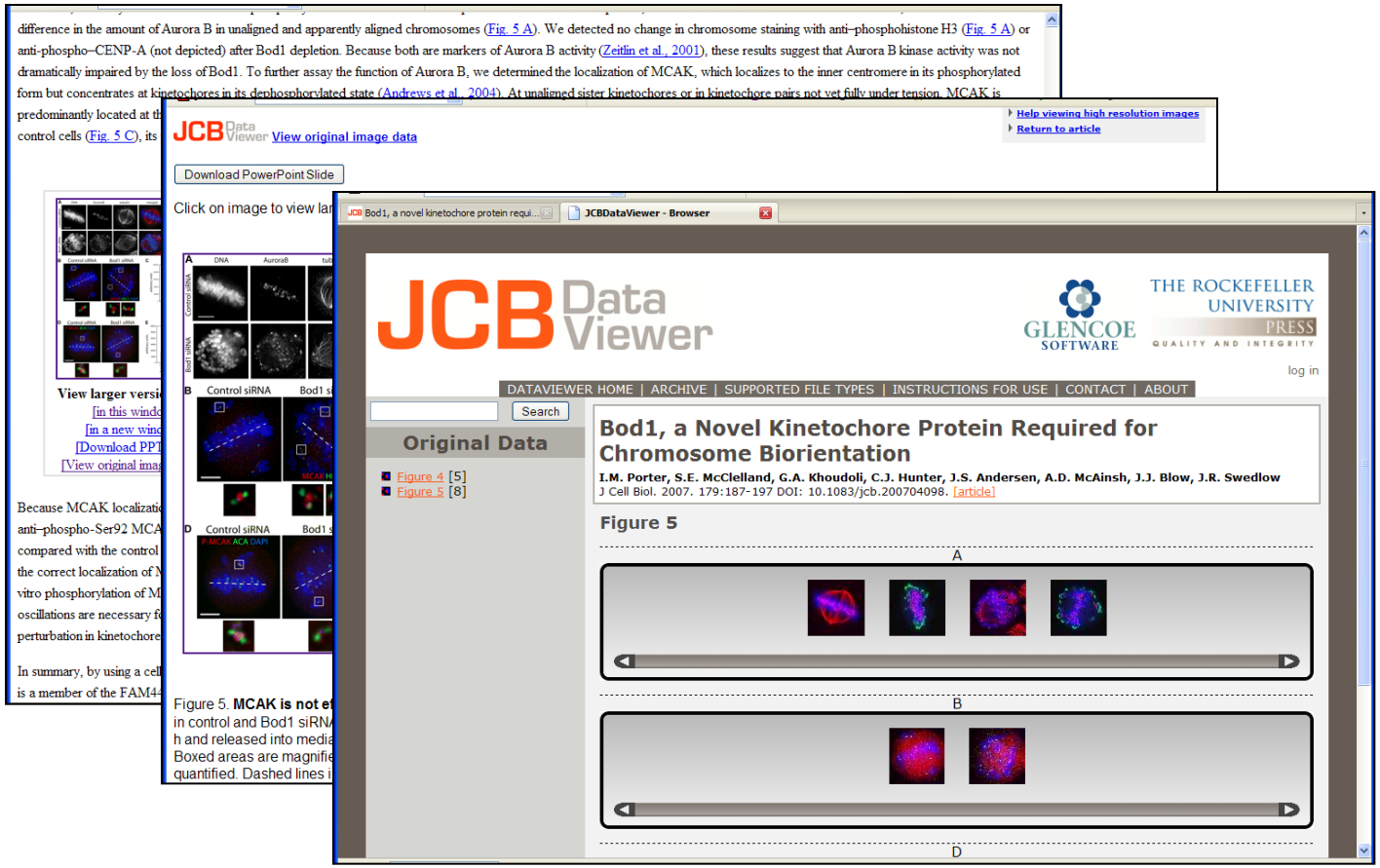

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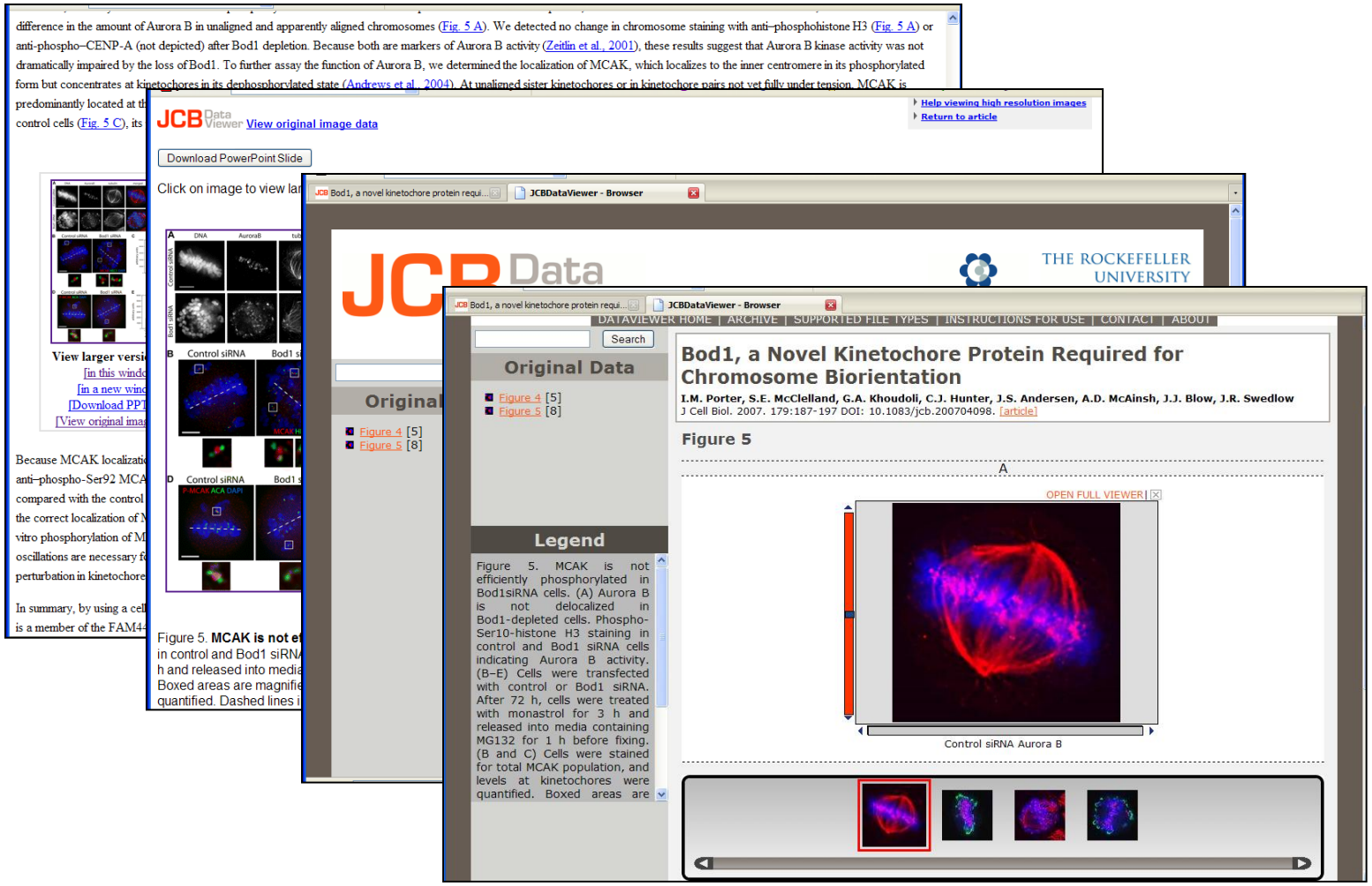

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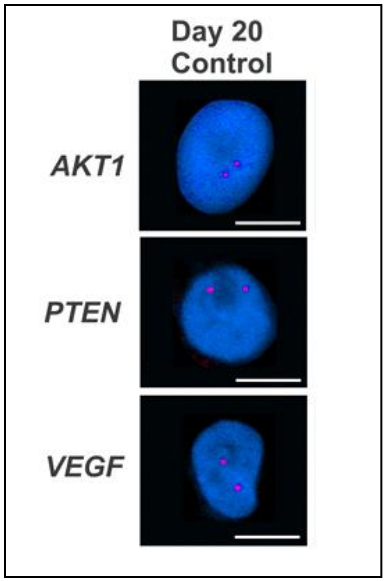
# Data Validation

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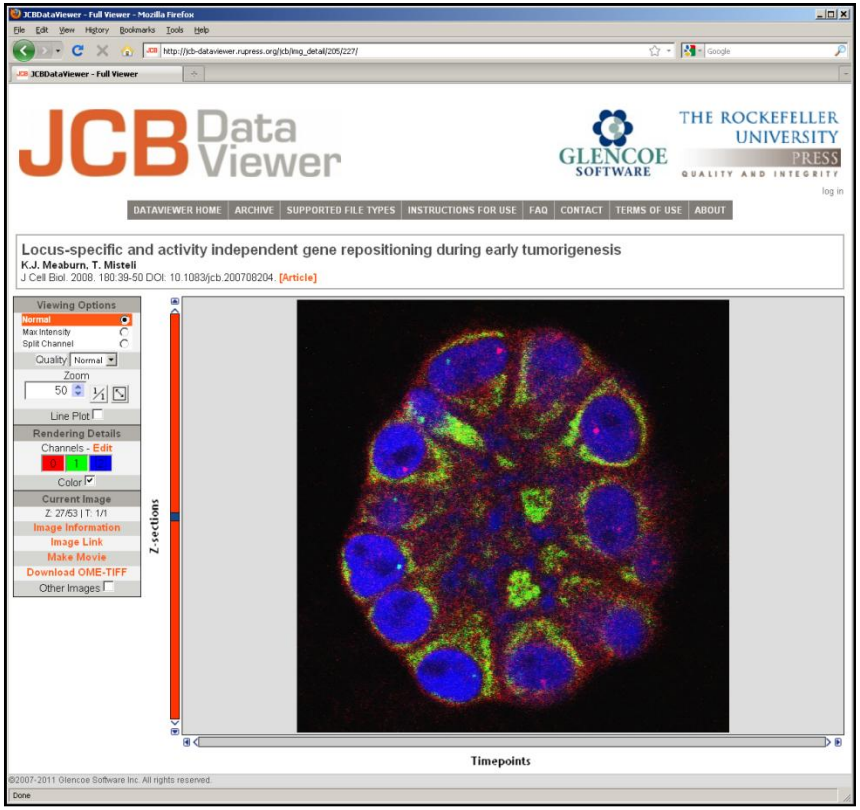

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**Voluntary participation rate (as of January, 2012):**

1.1 TB of image data (published and unpublished)

→221 published manuscripts

→ 780 published figures

→ 99,552 published images

→ 1,137,342 individual image frames

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# Where do we go from here?

- Continue to expand the range of data we can host.
- Continue to promote a new standard for sharing and archiving of published image data:
  - submission and publication of more than just 300 dpi, flat image data that editors, reviewers, and readers can only assume are fair representations of reality
  - public data archiving
  - greater openness in research
  - …an international repository for all published image data?

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*with special thanks to Emma Hill, Mike Rossner, and:*

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