

Introduction to digital images and ImageJ

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FAIIA

Outline

Visual perception

Digital image

Resolution and intensity sampling

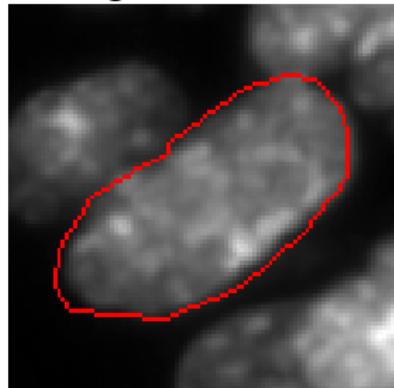
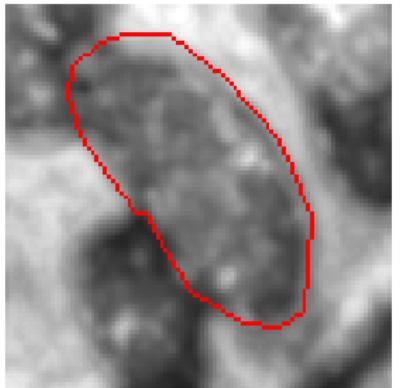
File format

Fiji/ImageJ

Visualization

A first pipeline for nuclei segmentation

Which nucleus is brighter?



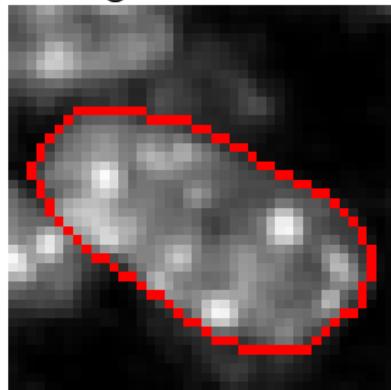
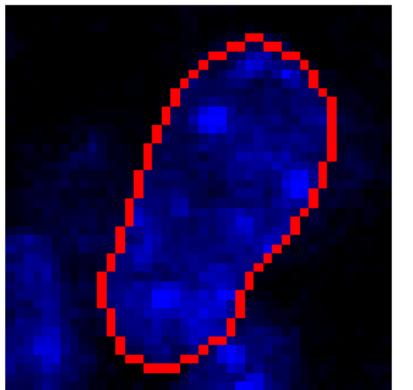
Can we trust the human eye?



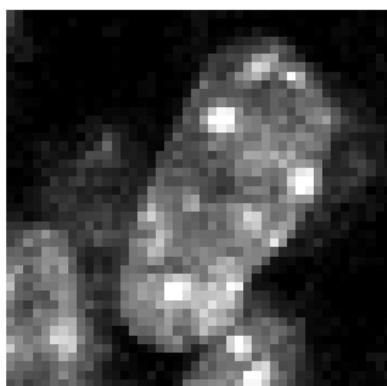
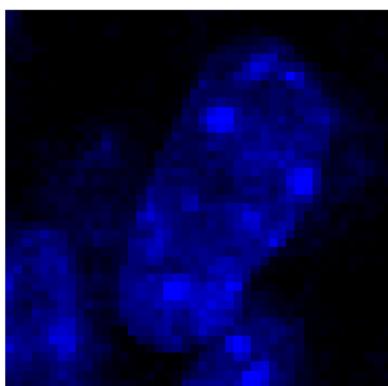
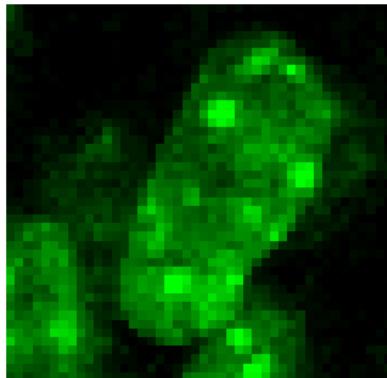
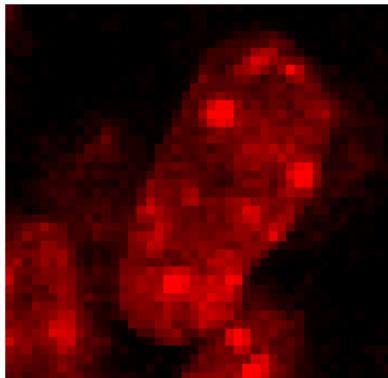
Can we trust the human eye?



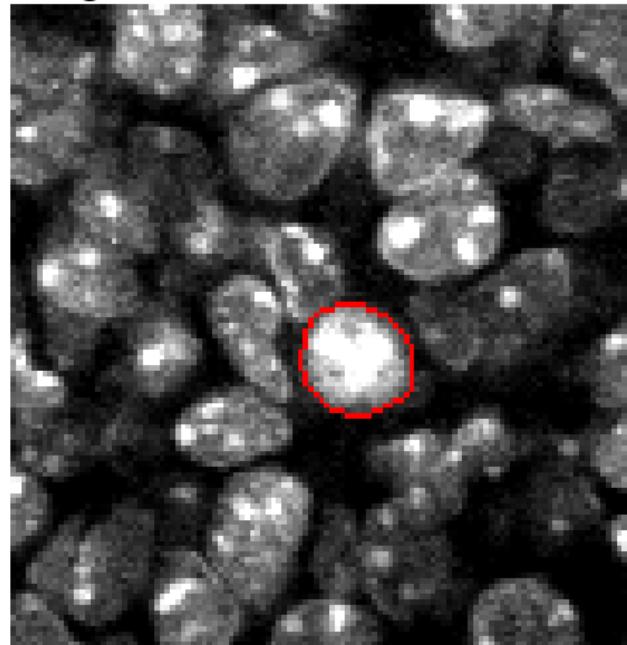
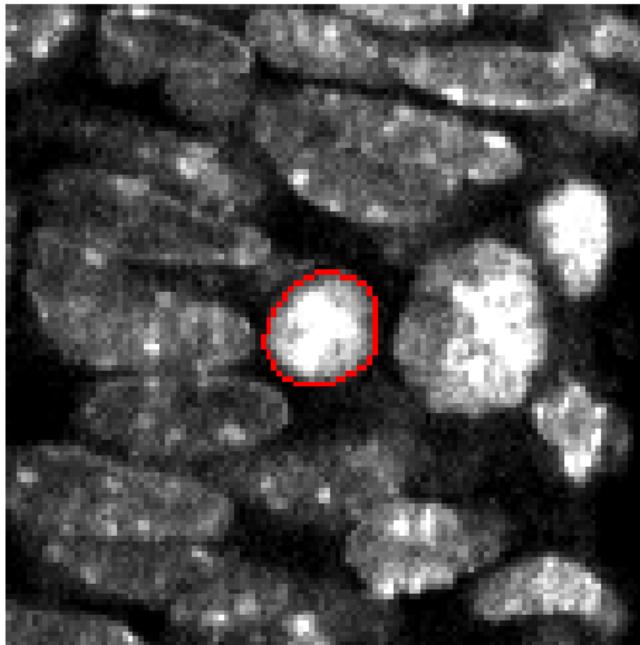
Which nucleus is brighter?



Can we trust the human eye?



Which nucleus is larger?



Can we trust the human eye?

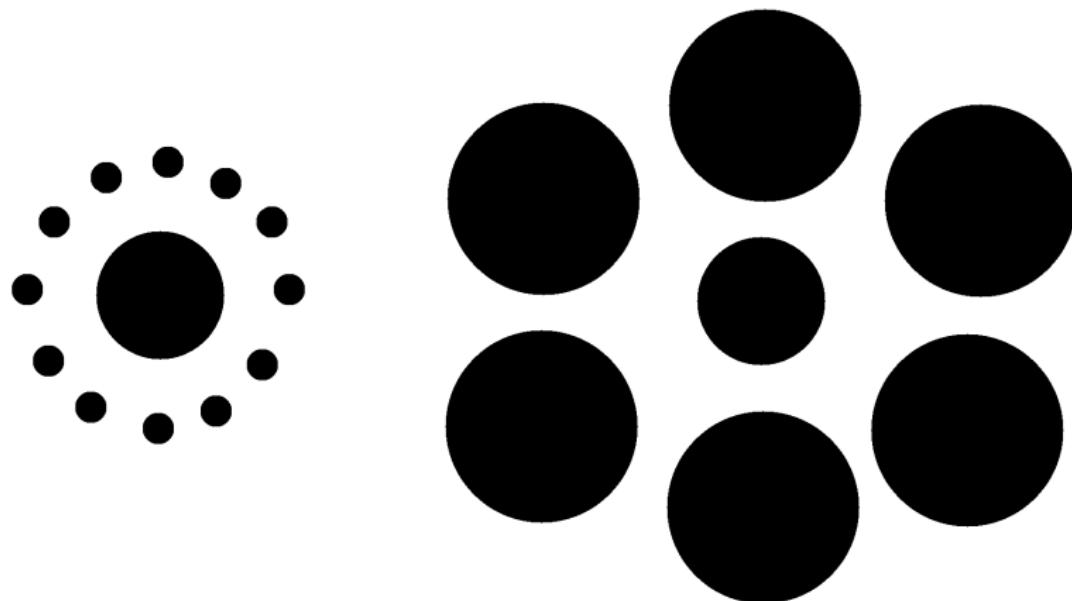


Image quantification

Image quantification is:

- **Unbiased**
- **Reproducible**
- **Fast**
- Appropriate to process a **very high number** of images

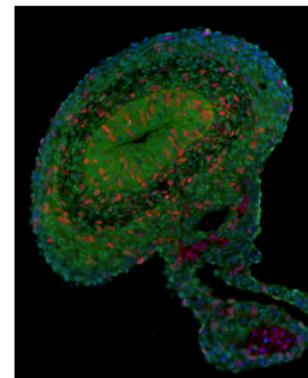
Object



Imaging system



Image



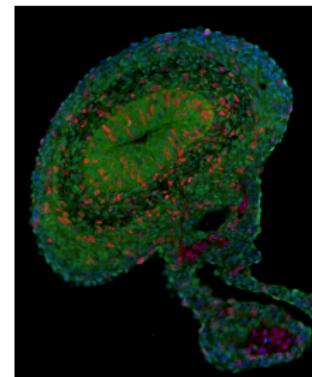
Object



Imaging system



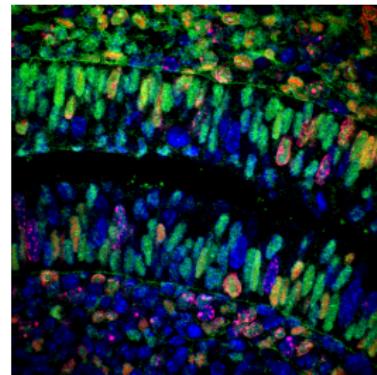
Image



Detection system



Digital image



Outline

Visual perception

Digital image

Resolution and intensity sampling

File format

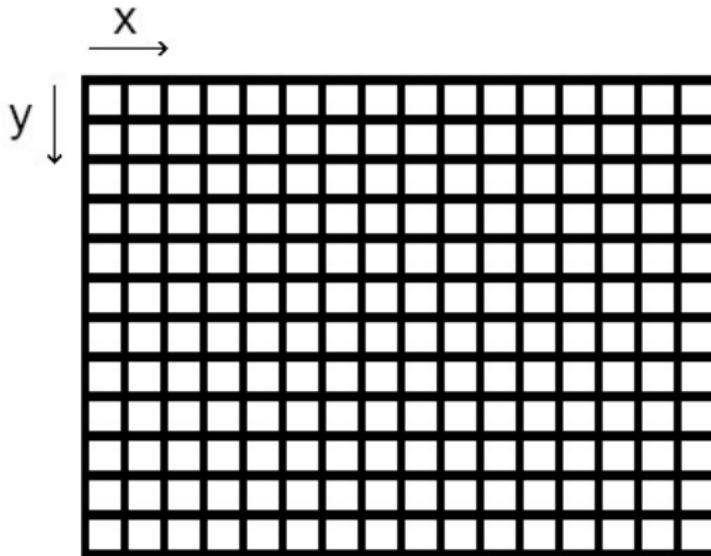
Fiji/ImageJ

Visualization

A first pipeline for nuclei segmentation

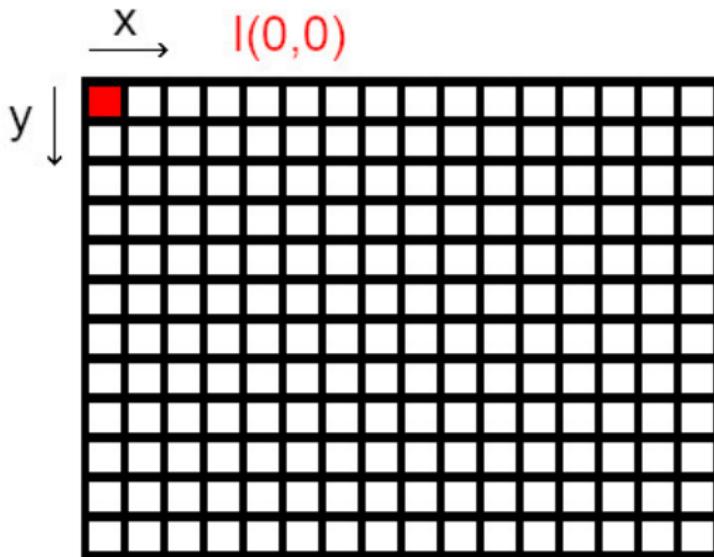
Digital image

A **digital image** is composed of **pixels** (picture elements)



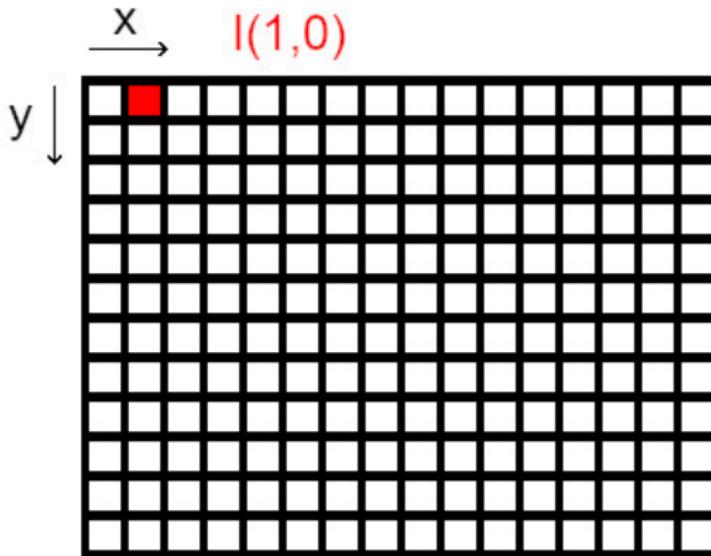
Digital image

Intensity observed at pixel of **coordinates** $x=0$ and $y=0$



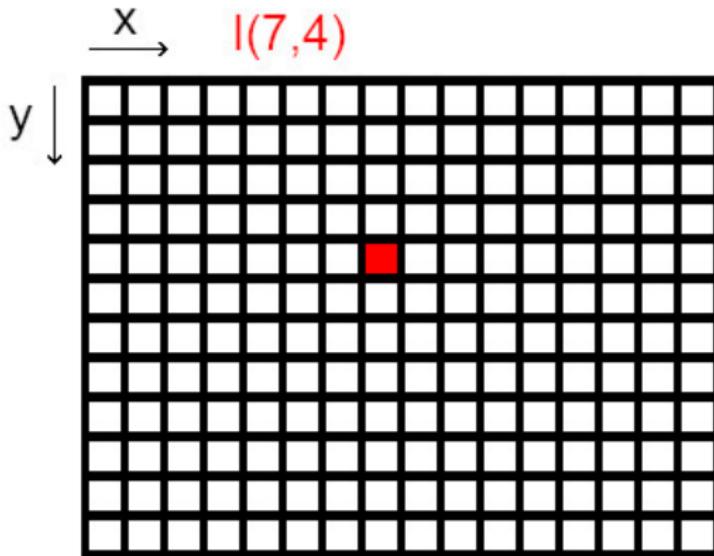
Digital image

Intensity observed at pixel of **coordinates** $x=1$ and $y=0$



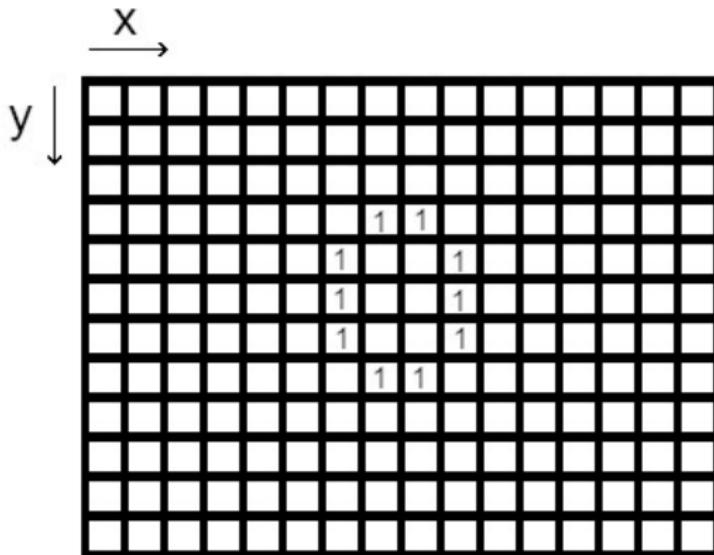
Digital image

Intensity observed at pixel of **coordinates** $x=7$ and $y=4$

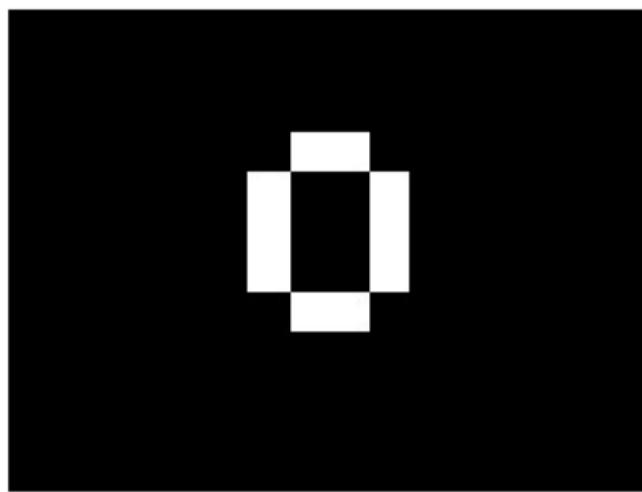


Digital image

Let us consider a **binary** image where 0 corresponds to black and 1 to white



Digital image



Digital image

- A **digital image** is a **rastered** image
- **Discrete intensity** are observed at each **pixel**
- A digital image can be viewed as a **matrix of intensities**
- **Mathematical operations** are applied to extract **salient information**

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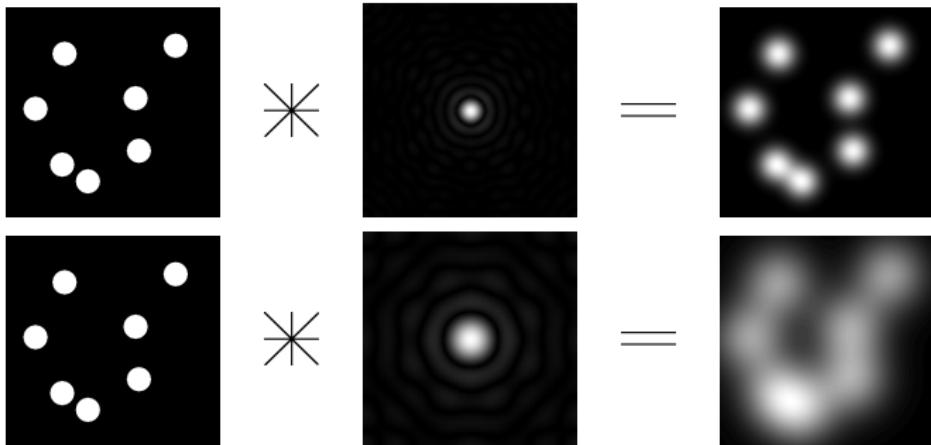
File format

Fiji/ImageJ

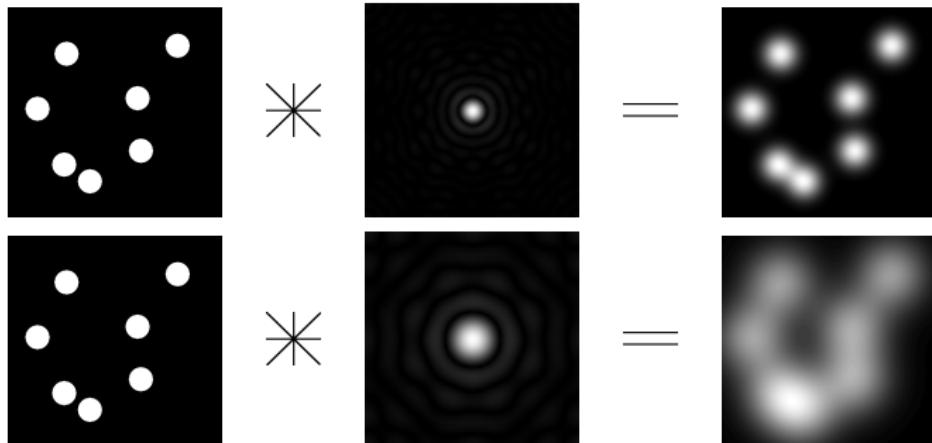
Visualization

A first pipeline for nuclei segmentation

Spatial sampling



Spatial sampling



The **Rayleigh criterion** gives the **minimal distance** to identify two close objects:

$$d = \frac{0.61\lambda}{n \sin(\theta)}$$

where λ is the wavelength and $n \sin(\theta)$ is the **numerical aperture**

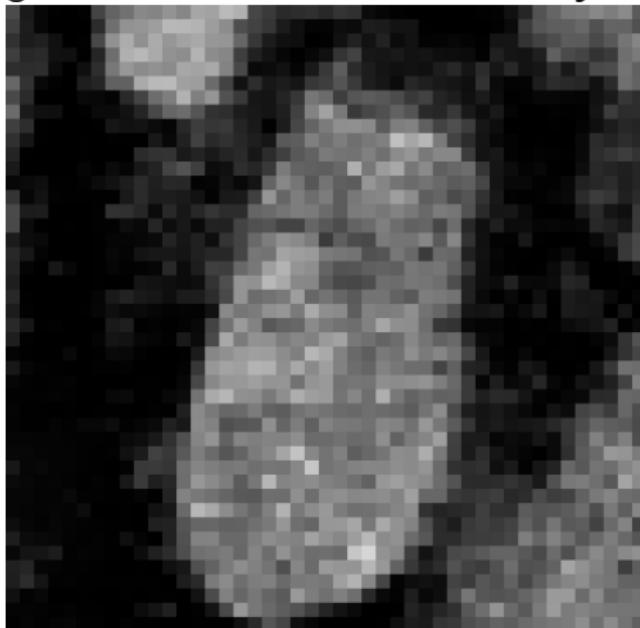
Intensity sampling

1 bit image: $2^1 = 2$ values for intensity at each pixel



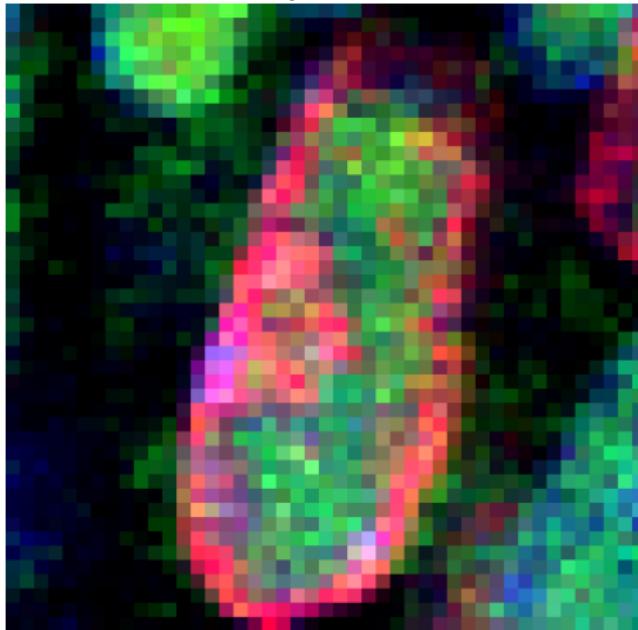
Intensity sampling

8 bits image: $2^8 = 256$ values for intensity at each pixel



Intensity sampling

24 bits image: $2^8 = 256$ **values for intensity** for the 3 channels at each pixel



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Proprietary format includes **metadata**:

- CZI (Carl Zeiss Images)
- NDPI (Hamamatsu)

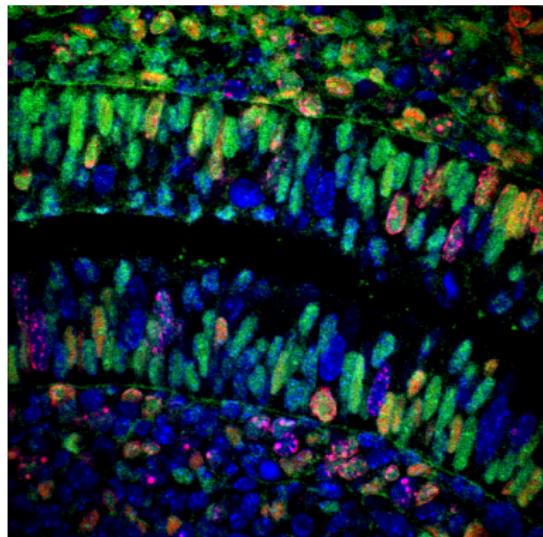
Lossless compression:

- TIFF (Tagged Image File Format), includes **metadata**
- PNG (Portable Network Graphic)
- HDF5 (Hierarchical Data Format), includes **metadata**
- OME-Zarr (NGFF), includes **metadata**

Lossy compression:

- JPEG (Joint Photographic Experts Group)

PNG



JPEG

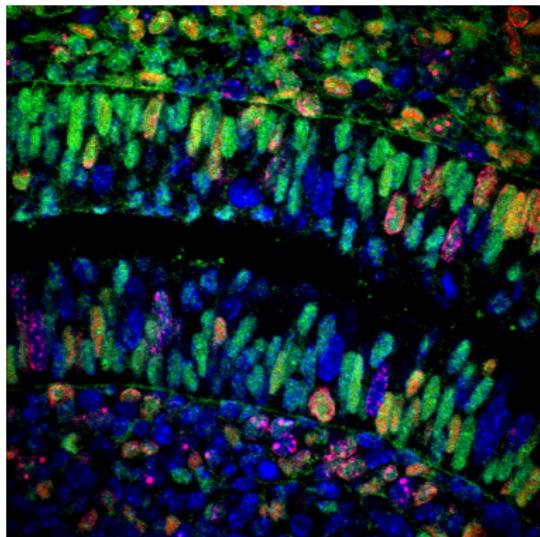
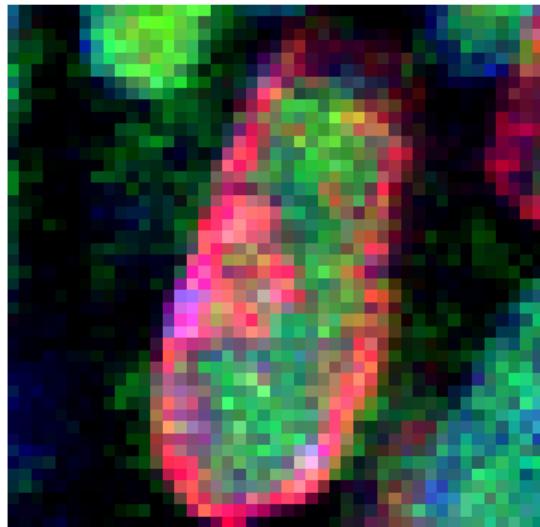
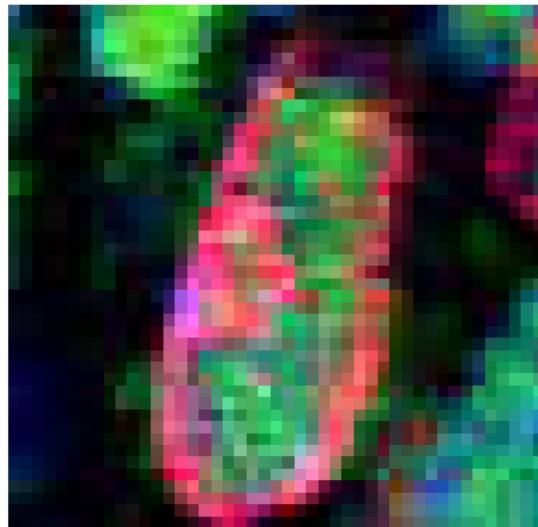


Image available at <https://doi.org/10.17632/5r6kf37zd4.1>

PNG



JPEG



12 bits image saved as PNG



12 bits image save as TIFF

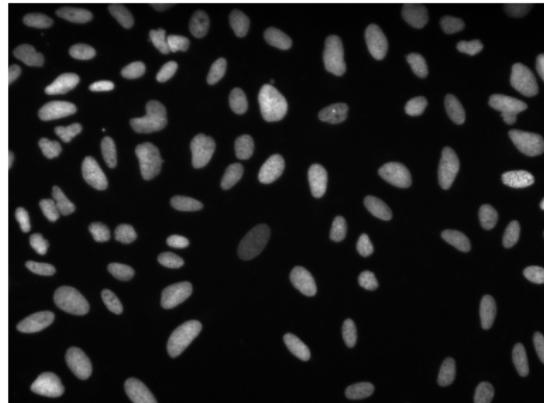


Image available at

<https://data.broadinstitute.org/bbbc/BBBC039/images.zip>

File format

- Store **raw data** in proprietary format
- Use images in proprietary format, TIFF, OME-Zarr for **analysis**
- Use PNG or JPEG images for **presentations**

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Fiji/ImageJ



Perspective | Published: 28 June 2012

Fiji: an open-source platform for biological-image analysis

Johannes Schindelin, Ignacio Arganda-Carreras, Erwin Frise, Verena Kaynig, Mark Longair, Tobias Pietzsch, Stephan Preibisch, Curtis Rueden, Stephan Saalfeld, Benjamin Schmid, Jean-Yves Tinevez, Daniel James White, Volker Hartenstein, Kevin Eliceiri, Pavel Tomancak & Albert Cardona

Nature Methods 9, 676–682 (2012) | Download Citation



Easy to Use

Fiji is easy to use and install—in one click, Fiji installs all of its plugins, features an automatic update, and includes comprehensive documentation.



Powerful

Fiji bundles together many popular and useful ImageJ plugins for image analysis into one integrated, user-extensible interface that eliminates dependencies and conflicts.



Free & Open Source

Like ImageJ itself, Fiji is an open source project hosted on GitHub, developed and written by the community.

Topic	Profiles	Views	Activity
Macro runs with no output ► ImageJ 1 version: 1.52, viewing, macro	4	27	13m
Problem with Ossy Script ► Development: 10, image, imagej, group	2	16	20m
Request for assistance in determining pipeline to threshold a low contrast image ► Analysis: 10, analysis, segmentation, thresholding	1	14	2h
Saving BigDataViewer Slices into bigdataviewer.hdf5 ► Development: 10, image, imagej, hdf5	5	28	3h
QIJOIUser: loading to arrayviewer/bounds/description ► ImageJ 1 version: 1.52, viewing, macro, mrc	1	17	5h
Automated and Fiji Legacy ► ImageJ 1 version: 1.52, 1.53, 1.54, 1.55, legacy, macro, scripting	1	14	5h
Get/Save a macro from a macro ► ImageJ 1 version: 1.52, 1.53, 1.54, 1.55	4	48	5h
Which Fiji plugins should be featured on the front page? ► Release: 10, image	10	800	8h
Read one-end metadata ► Image Analysis: 10, bioformats, image, plugin, macro	2	10	10h
Radial Intensity Distribution in FijiImageJ ► Image Analysis: 10, image	2	13	10h
Registration help ► Image Analysis: 10, image, registration	4	20	12h

Practice

<https://youtu.be/wCi5mypkZko>

<https://youtu.be/rYaJRf0Ptw8>

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Histogram



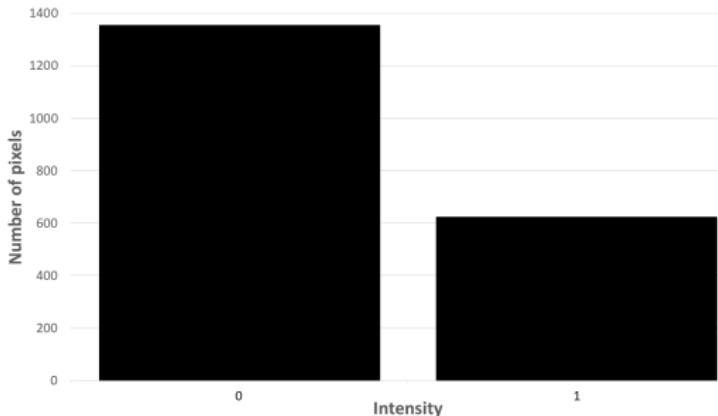
Histogram

1356 pixels with intensity equal to **0** and **624 pixels** with intensity equal to **1**

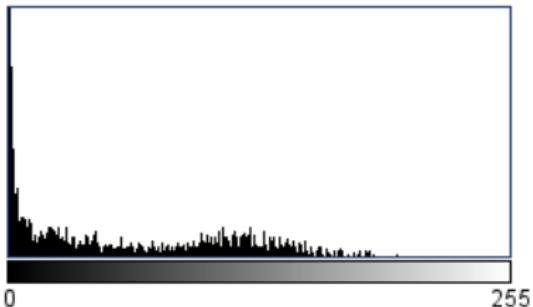
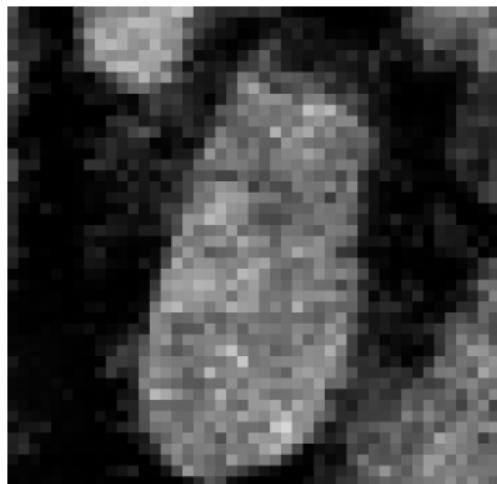


Histogram

1356 pixels with intensity equal to **0** and **624 pixels** with intensity equal to **1**



Histogram



N: 1980

Mean: 61.328

StdDev: 54.535

Value: 167

Min: 0

Max: 214

Mode: 0 (153)

Count: 1

Visualization

https://youtu.be/m_ZPoV4QONk

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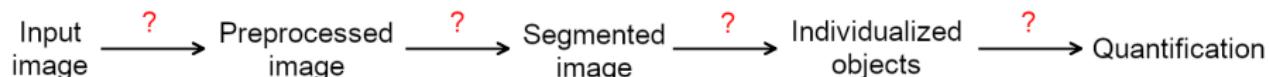
Fiji/ImageJ

Visualization

A first pipeline for nuclei segmentation

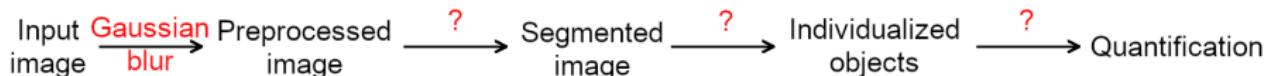
Pipeline

Simple pipeline to segment nuclei



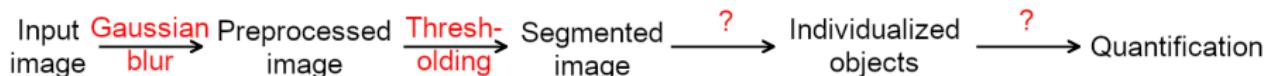
Pipeline

Simple pipeline to segment nuclei



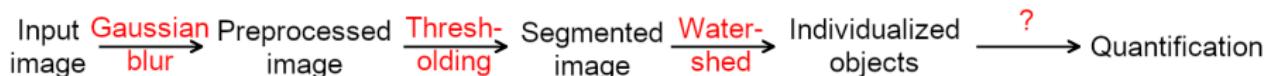
Pipeline

Simple pipeline to segment nuclei



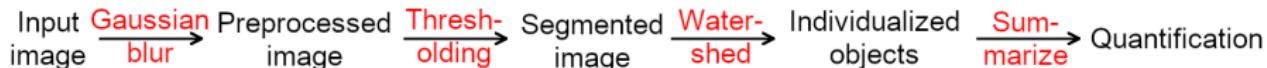
Pipeline

Simple pipeline to segment nuclei



Pipeline

Simple pipeline to segment nuclei



Practice

<https://youtu.be/4i6UTUqyUkg>