

# Introduction to digital images and ImageJ

Thierry Pécot  
Associate Researcher  
CZI Imaging Scientist

# Outline

Visual perception

Digital image

Resolution and intensity sampling

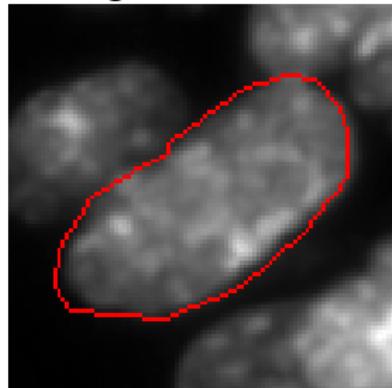
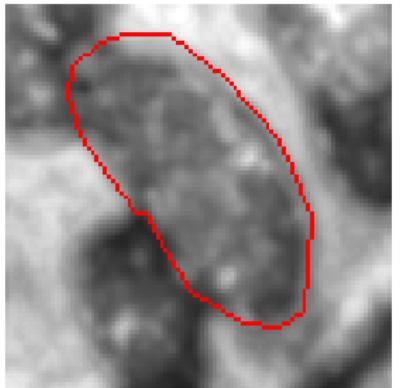
File format

Fiji/ImageJ

Visualization

A first pipeline for nuclei segmentation

Which nucleus is brighter?



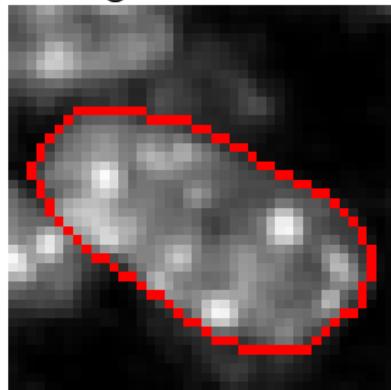
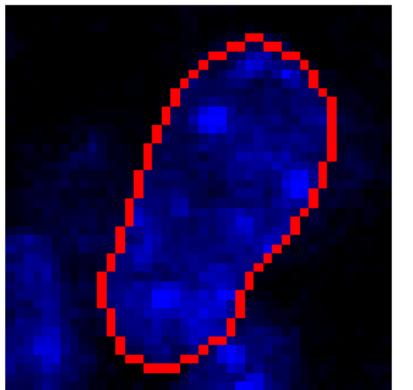
# Can we trust the human eye?



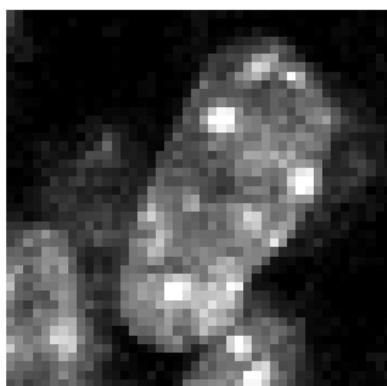
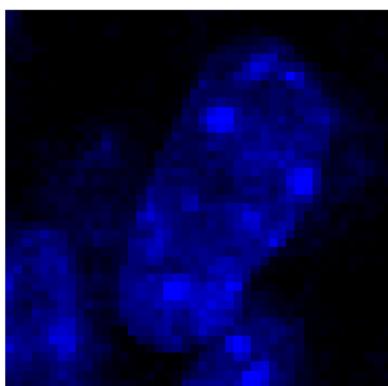
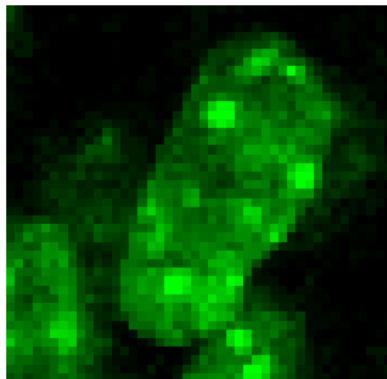
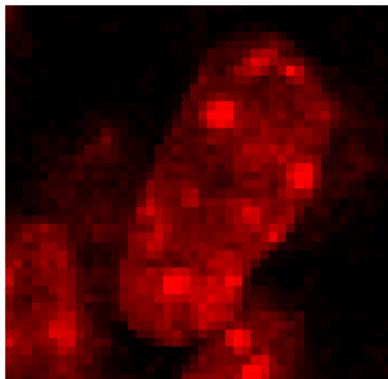
# Can we trust the human eye?



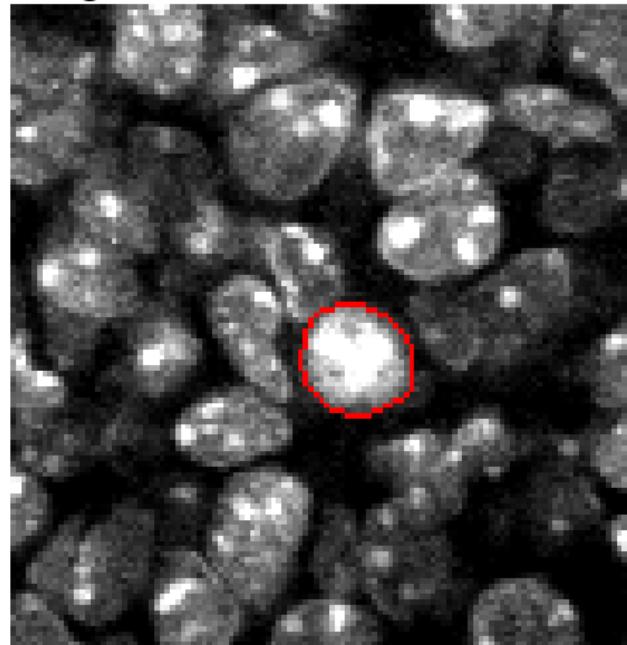
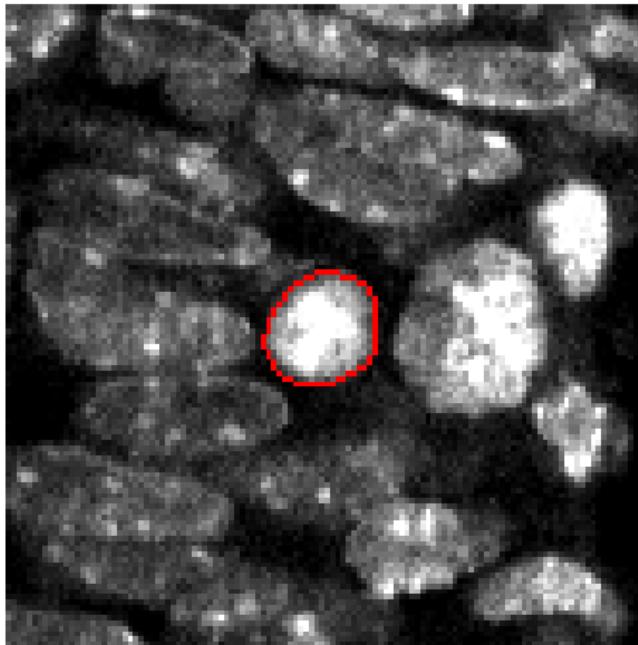
Which nucleus is brighter?



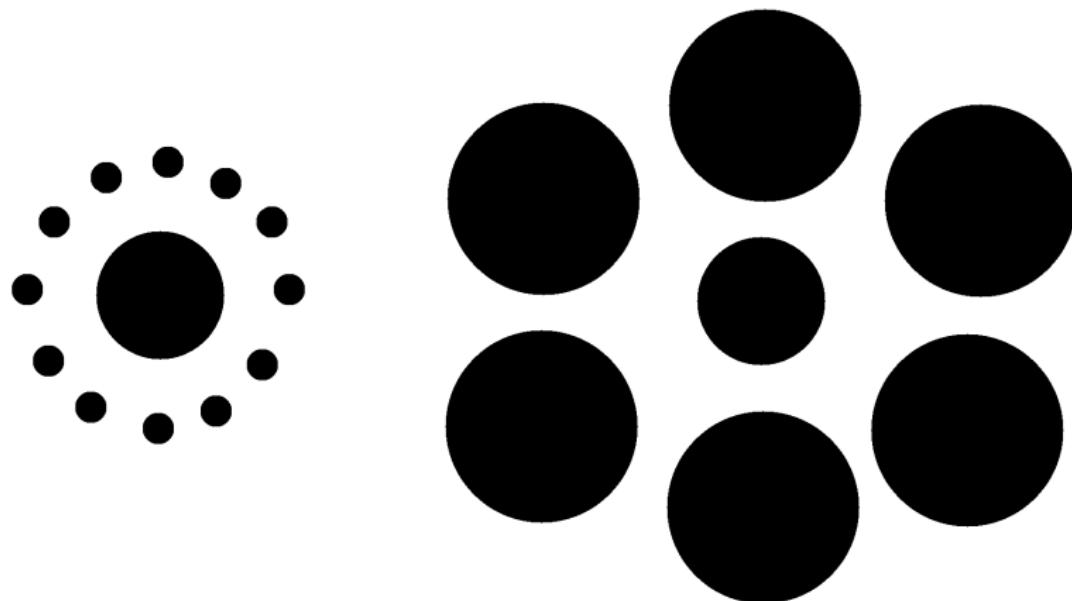
# Can we trust the human eye?



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

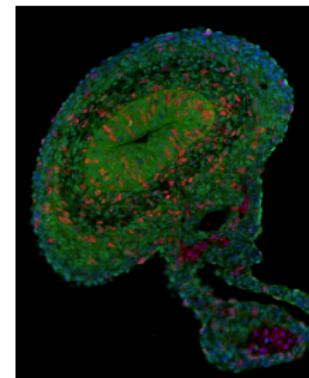
Object



Imaging system



Image



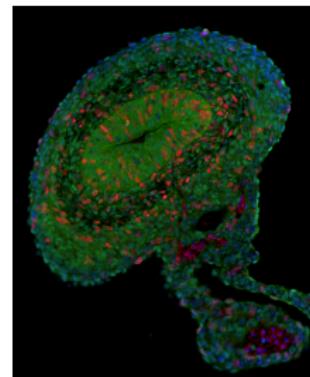
Object



Imaging system



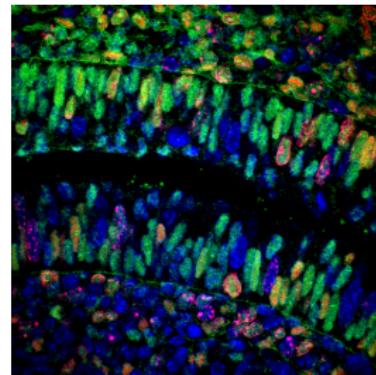
Image



Detection system



Digital image



# Outline

Visual perception

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Resolution and intensity sampling

File format

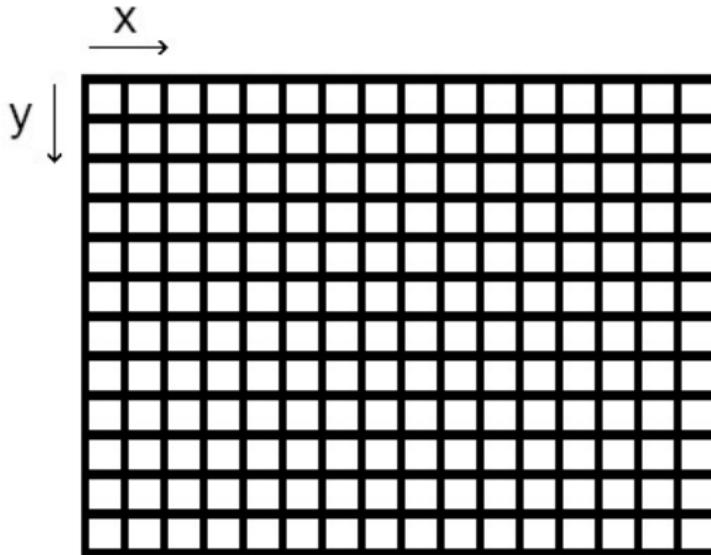
Fiji/ImageJ

Visualization

A first pipeline for nuclei segmentation

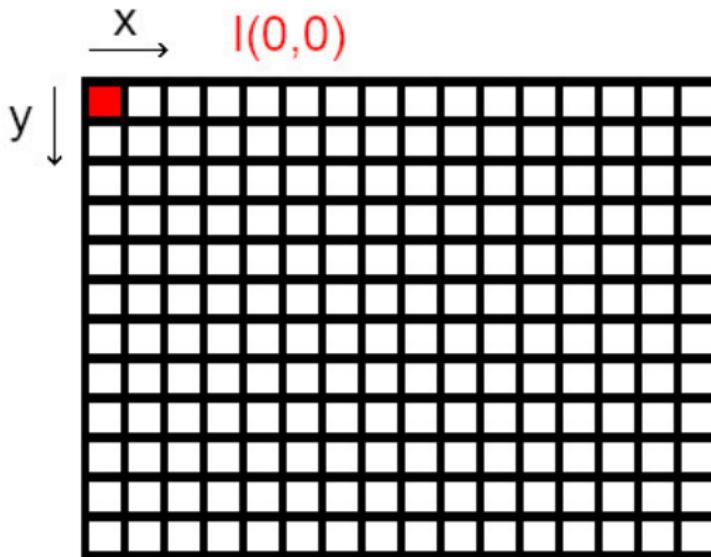
# Digital image

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



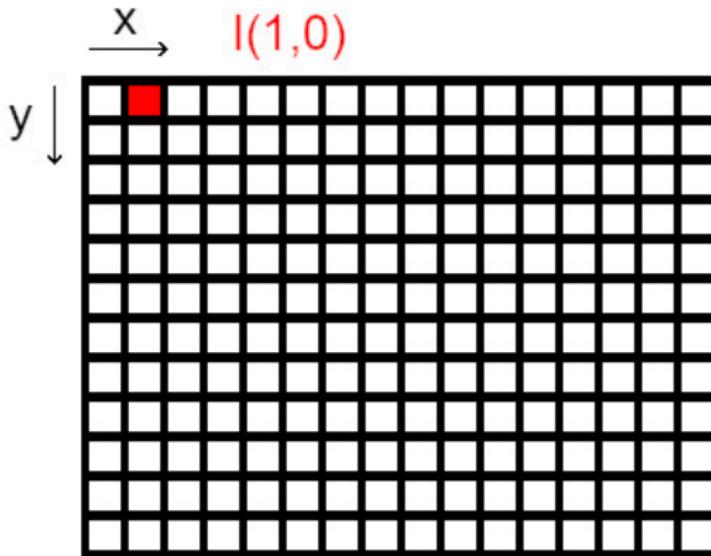
# Digital image

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



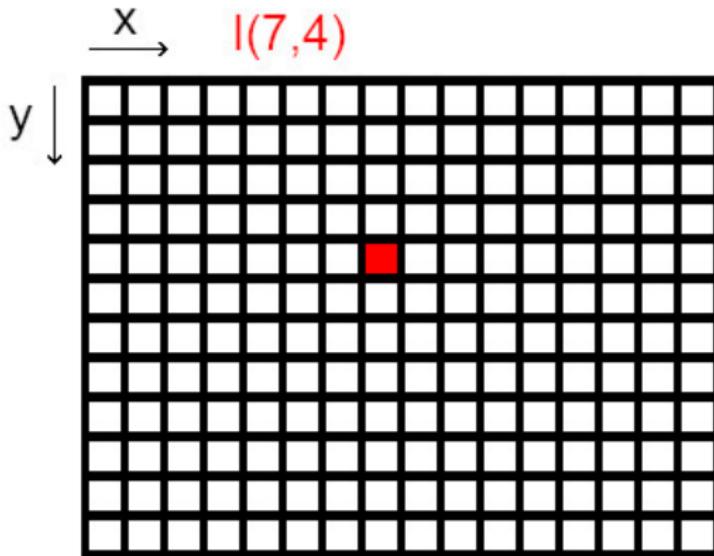
# Digital image

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



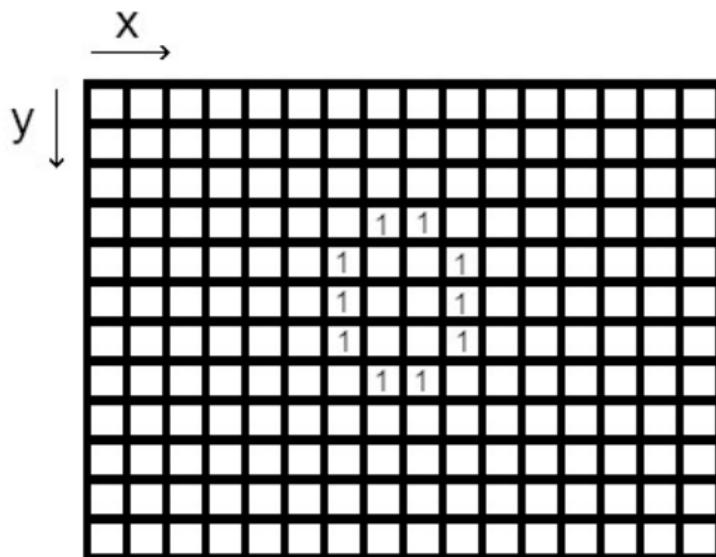
# Digital image

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

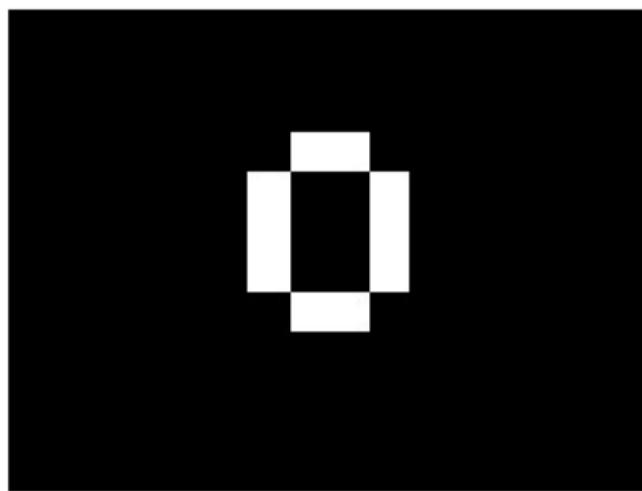


# Digital image

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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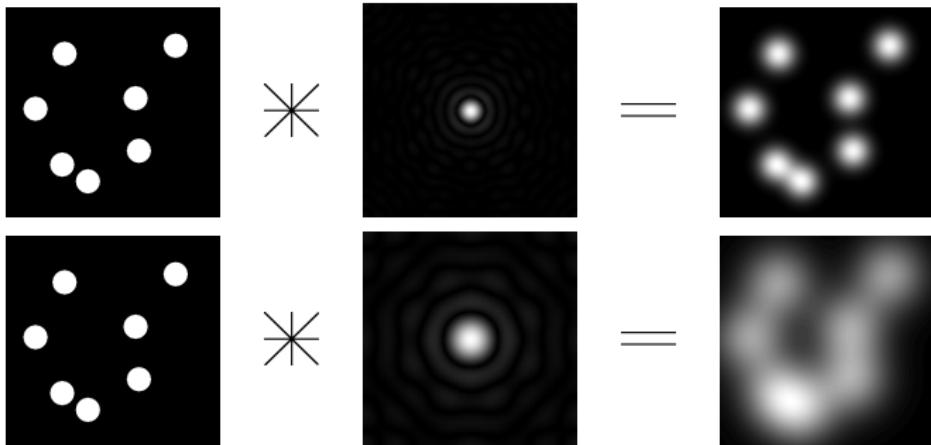
File format

Fiji/ImageJ

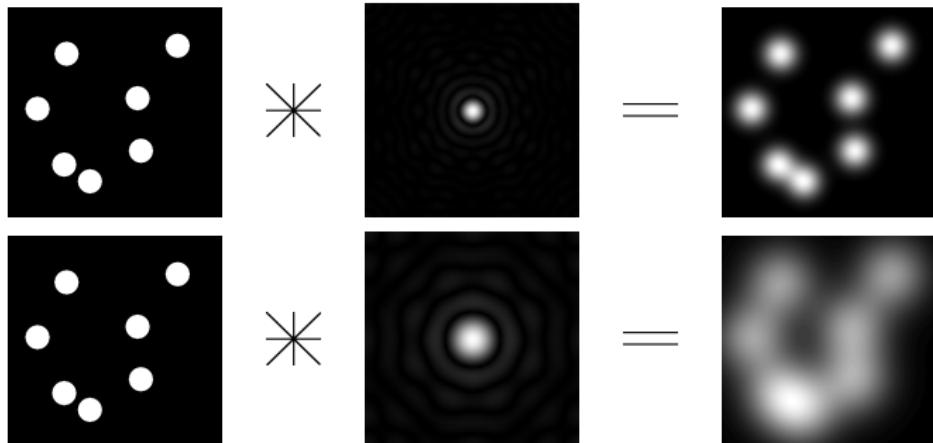
Visualization

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

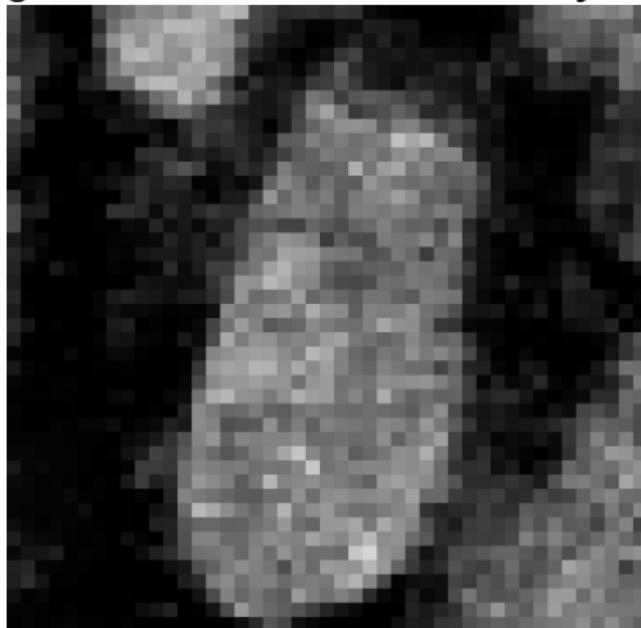
## Intensity sampling

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



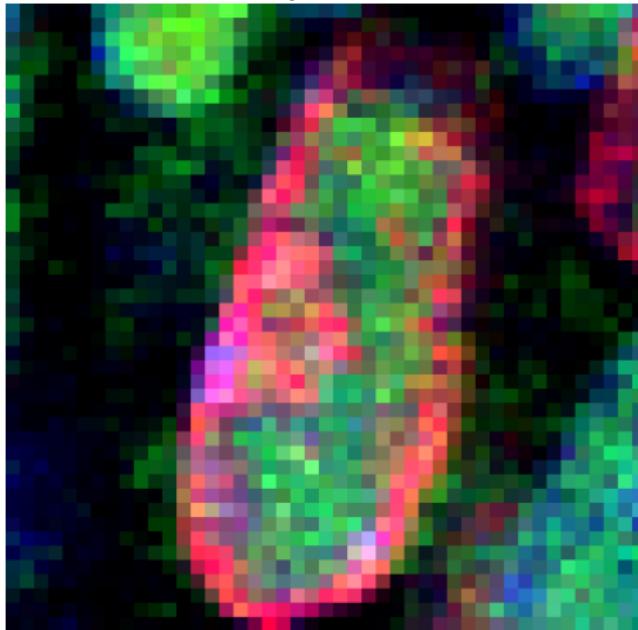
## Intensity sampling

**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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**File format**

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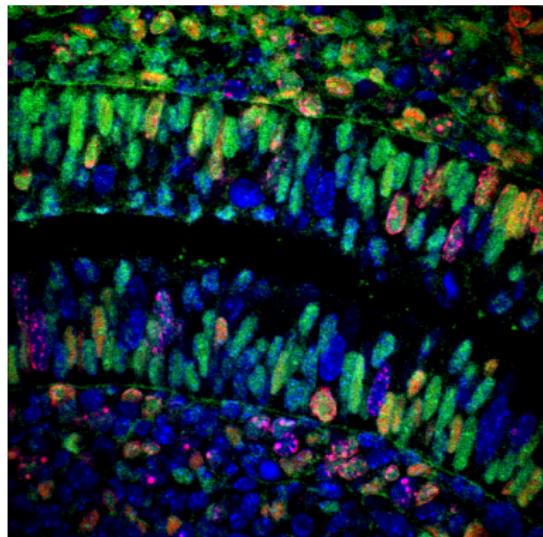
## Lossless compression:

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

## Lossy compression:

- JPEG (Joint Photographic Experts Group)

PNG



JPEG

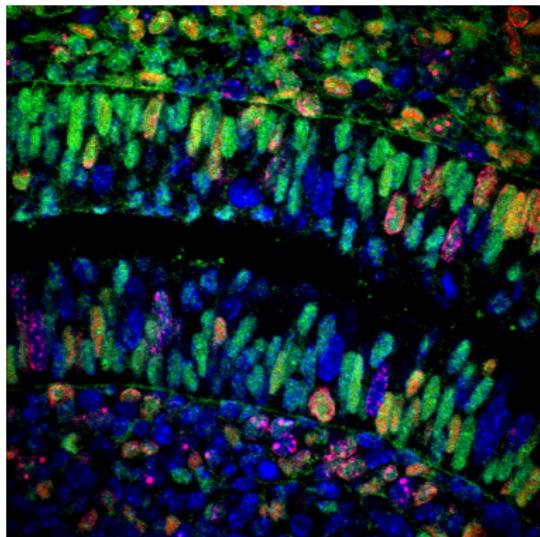
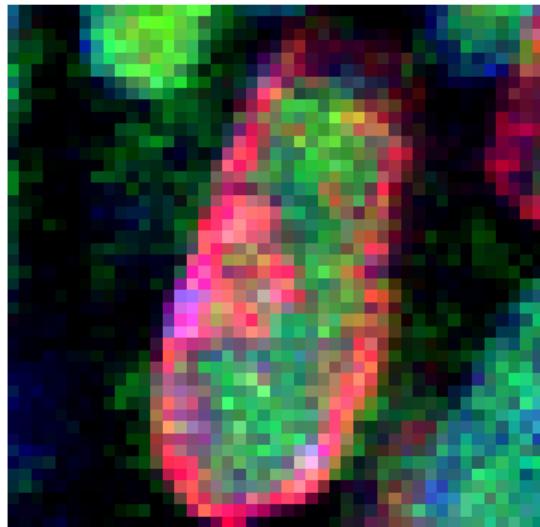
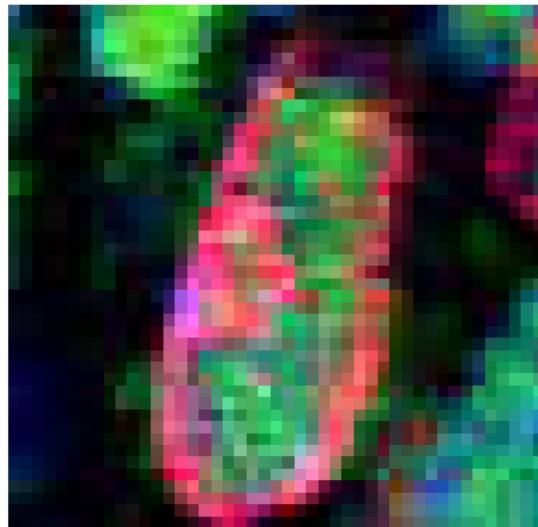


Image available at <https://doi.org/10.17632/5r6kf37zd4.1>

PNG



JPEG



12 bits image saved as PNG



12 bits image save as TIFF

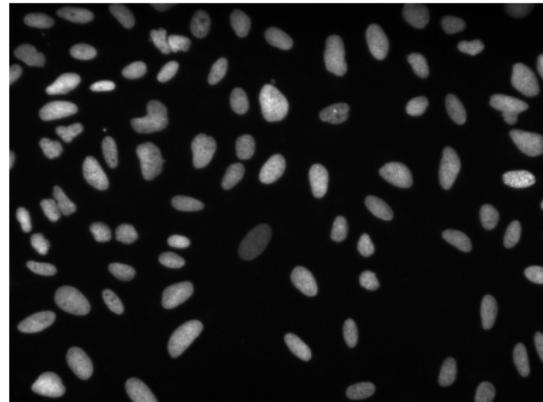


Image available at

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

# File format

- Store **raw data**
- Use TIFF images 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.

### Why Fiji?



### Easy to Use

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

### Powerful

Fiji bundles together many popular and useful ImageJ plugins for image analysis into one integrated, user-friendly 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 Ossuary 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
Q3DView loading to anaylyzeviewer/boundariescription ► ImageJ 1 version: 1.52, viewing, macro, 3d	1	17	5h
Automated and Fiji Legacy ► ImageJ 1 version: 1.52, 1.53, 1.54, legacy, imagej, legacy	1	14	5h
Get rid of a macro from a macro ► ImageJ 1 version: 1.52, 1.53, 1.54, legacy, imagej, legacy	4	48	5h
Which Fiji plugins should be featured on the front page? ► Release: 10, imagej	10	800	8h
Read one-end metadata ► Image Analysis: 10, bioformats, imagej, plugin, macro	2	10	10h
Radial Intensity Distribution in FijiImageJ ► Image Analysis: 10, imagej	2	13	10h
Registration help ► Image Analysis: 10, imagej, registration	4	20	12h

# Practice

<https://youtu.be/wCi5mypkZko>

<https://youtu.be/rYaJRf0Ptw8>

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# Histogram



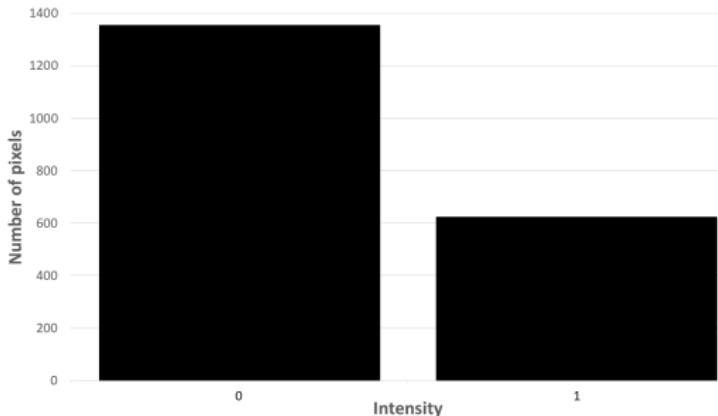
# Histogram

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

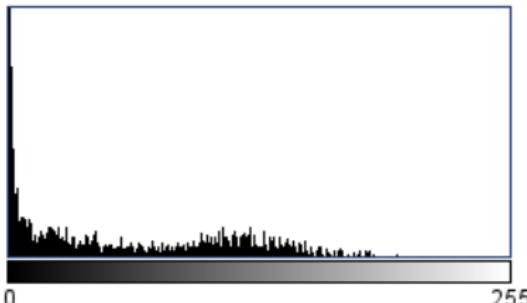
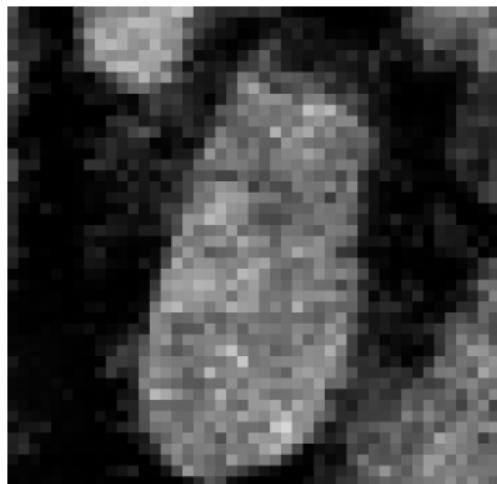


# Histogram

**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](https://youtu.be/m_ZPoV4QONk)

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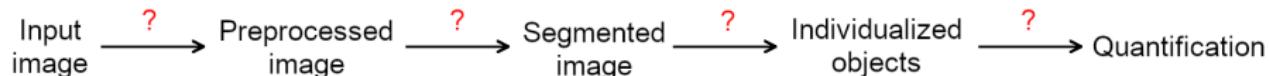
Fiji/ImageJ

Visualization

**A first pipeline for nuclei segmentation**

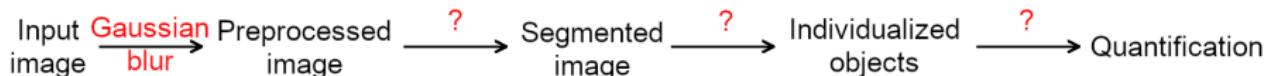
# Pipeline

## Simple pipeline to segment nuclei



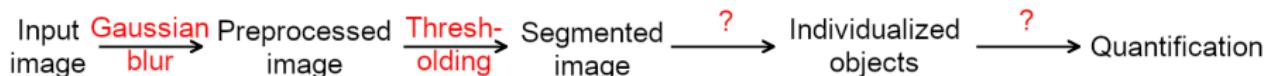
# Pipeline

## Simple pipeline to segment nuclei



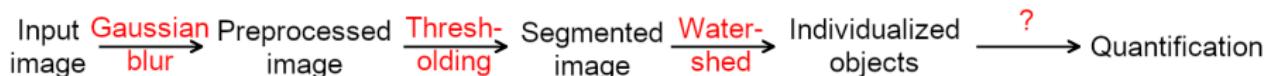
# Pipeline

## Simple pipeline to segment nuclei



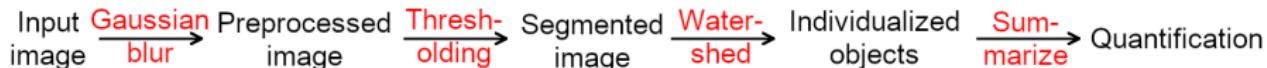
# Pipeline

## Simple pipeline to segment nuclei



# Pipeline

## Simple pipeline to segment nuclei



# Practice

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