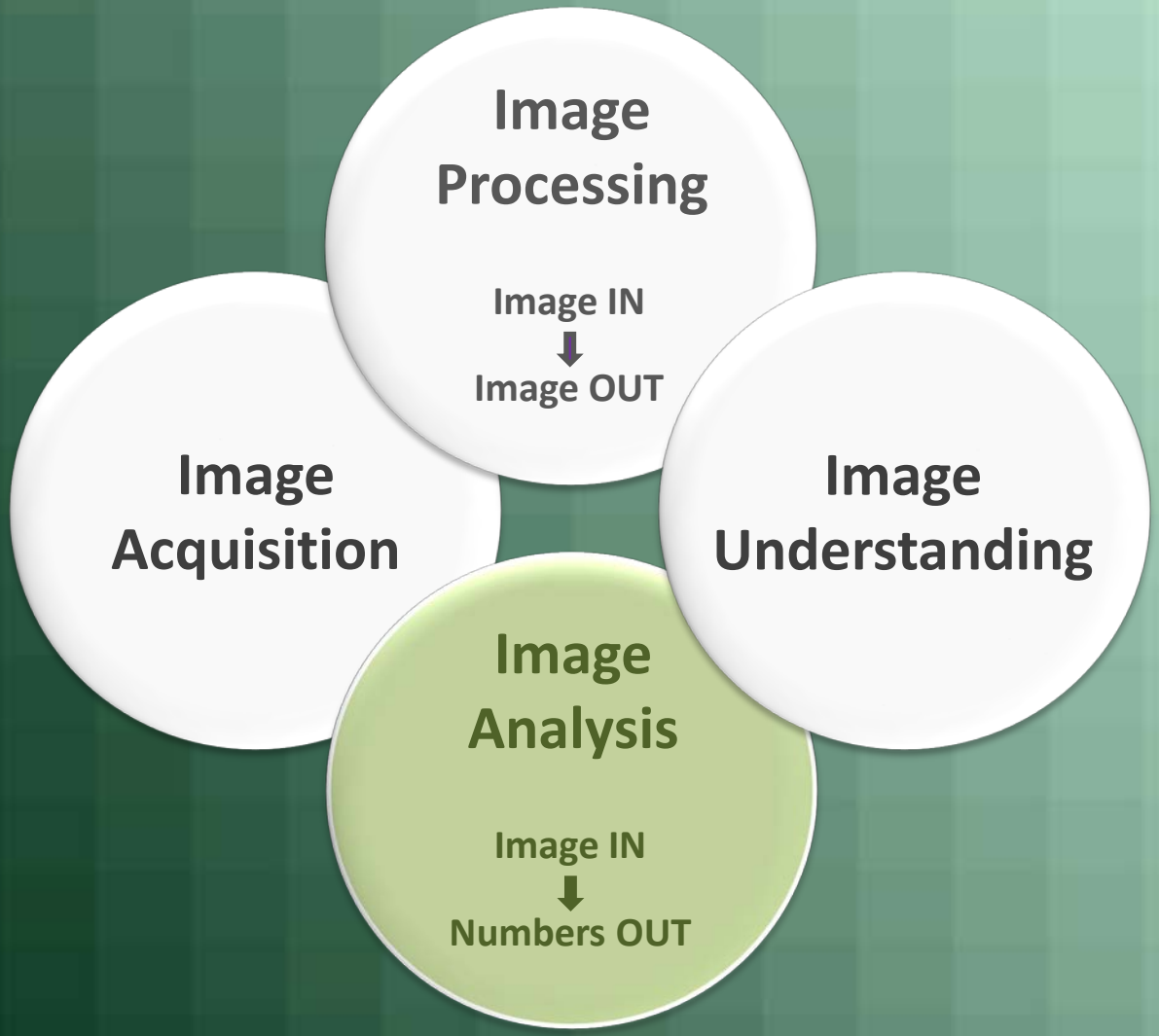


Image Processing
Image IN
Image OUT
Image Acquisition
Image Understanding
Image Analysis
Image IN
Numbers OUT

Measurement

Basics

Where to measure?

Where?

- the whole image

-at a specific point

- in a region

- within a threshold

- in an object

What to measure?

What?

- Position
- Distance
- Number
- Extend
- Shape
- Intensity

Position

Position:

-*absolute*: xyzt coordinate of a point

x=0 left
y=0 top
z=0 no clear definition
t=0 first recorded time point

Watch out for calibration
know what you are measuring!!

-*relative*: xyzt distance to reference

to image boarder
to reference point
to other time point
to other object (e.g. to nucleus)

Calibration

-Stored in image header AND read out correctly

- stored in image header but NOT read out

- known from acquisition settings
    - pixel size of camera / total magnification used
    - information in confocal images
    - use ruler – calibrate (stereo microscope)

Position

Position:

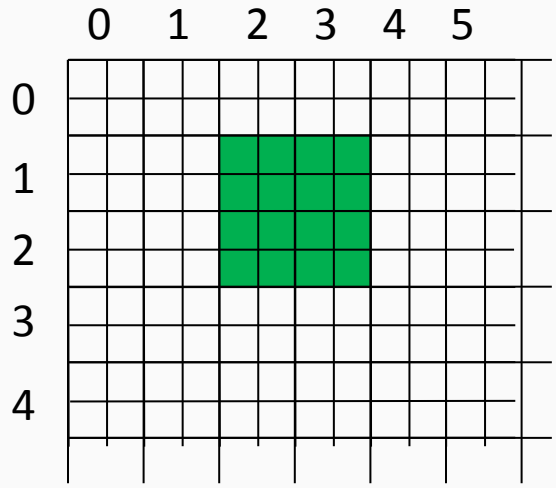
-*absolute*: xyzt coordinate of **an object**

x=0 left
y=0 top
z=0 no clear definition
t=0 first recorded time point

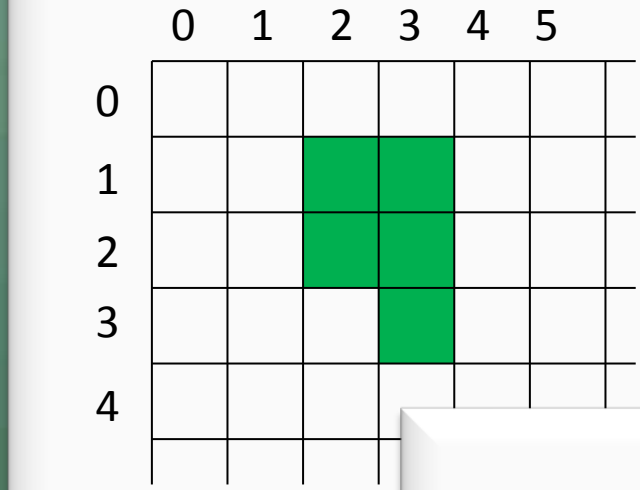
-*relative*: xyzt distance to reference

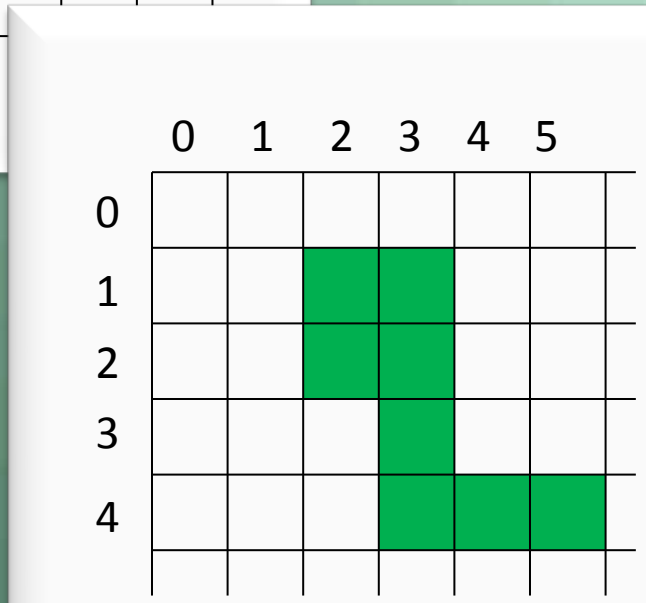
to image boarder
to reference point
to other time point
to other object (e.g. to nucleus)

Position

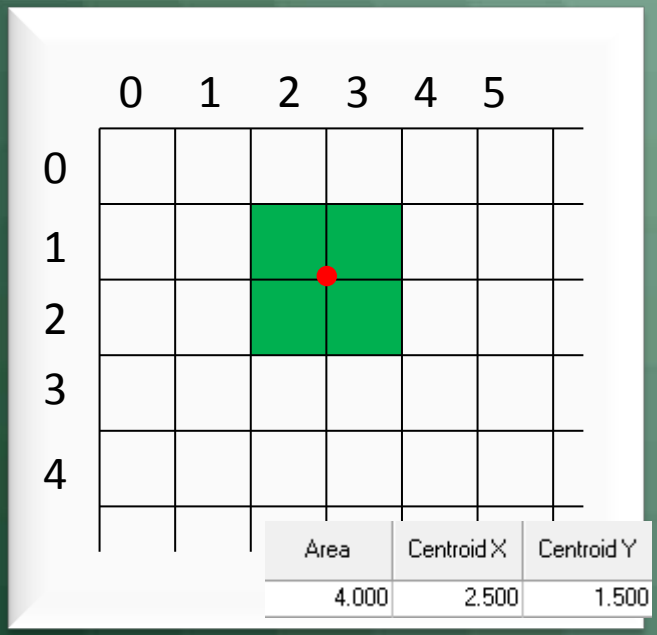


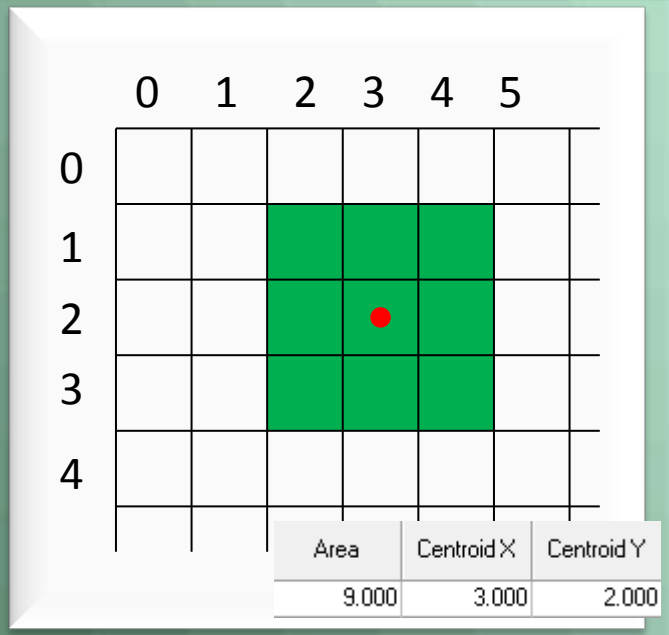
Center = x 2.5 y 1.5

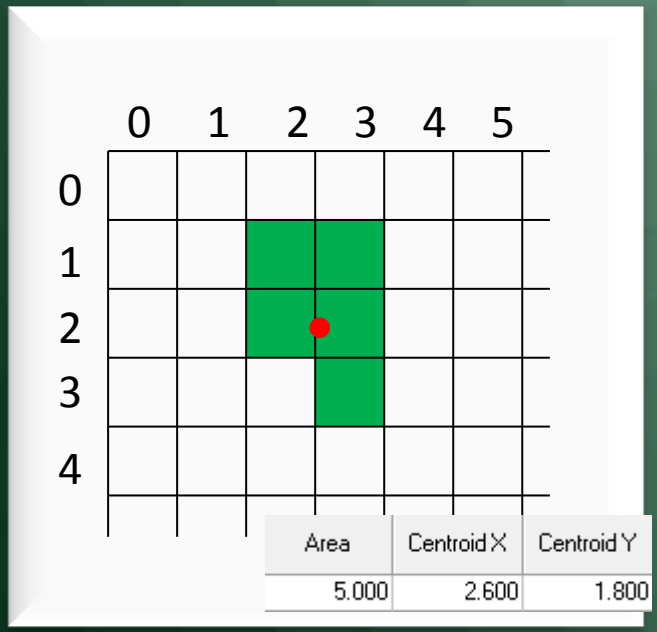


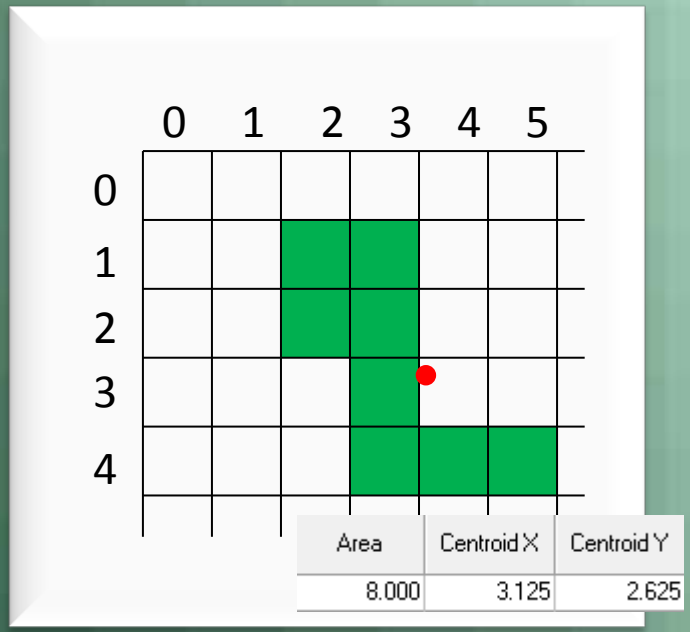


Position


0 1 2 3 4 5
0
1
2
3
4
Area
Centroid X
Centroid Y
4.000
2.500
1.500


0 1 2 3 4 5
0
1
2
3
4
Area
Centroid X
Centroid Y
9.000
3.000
2.000


0 1 2 3 4 5
0
1
2
3
4
Area
Centroid X
Centroid Y
5.000
2.600
1.800

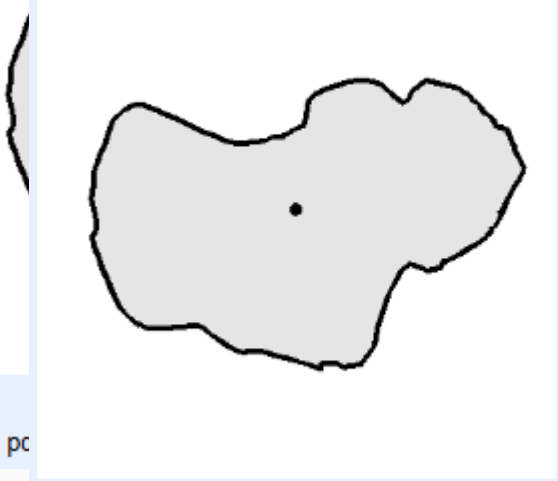

0 1 2 3 4 5
0
1
2
3
4
Area
Centroid X
Centroid Y
8.000
3.125
2.625

Position

Metamorph

Centroid

Centroid X and Y



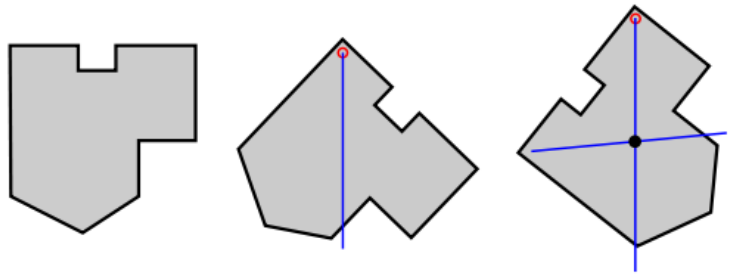
The pc

The X and Y coordinates of the centroid of the object.

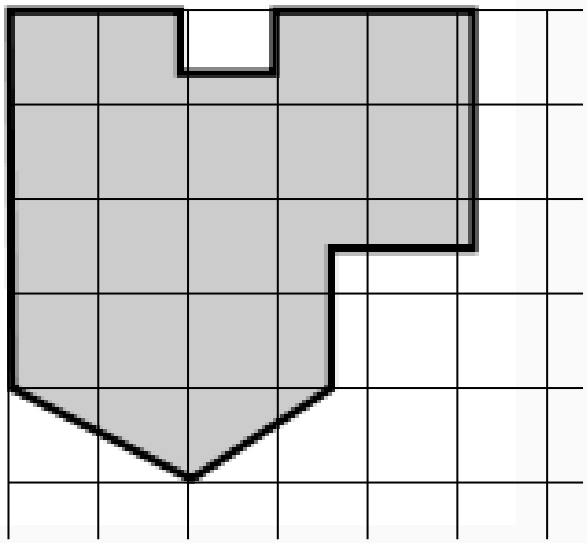
Wikipedia

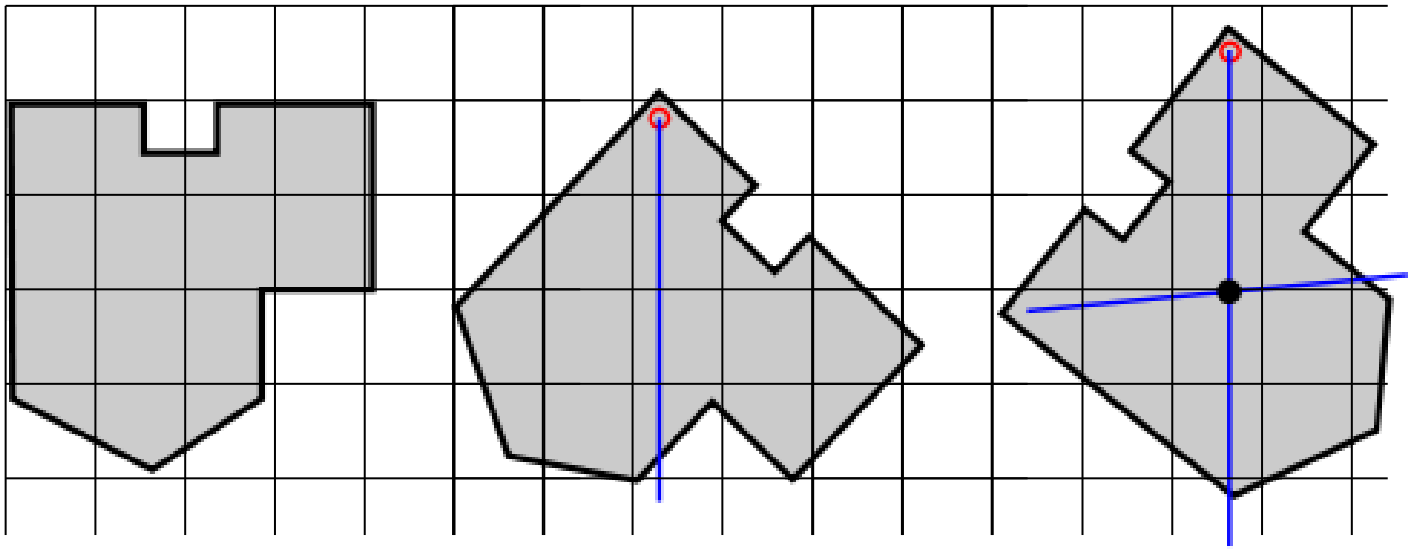
2 Locating the centroid

- 2.1 Plumb line method
- 2.2 Balancing Method
- 2.3 Of a finite set of points
- 2.4 By geometric decomposition
- 2.5 By integral formula



Position





Position

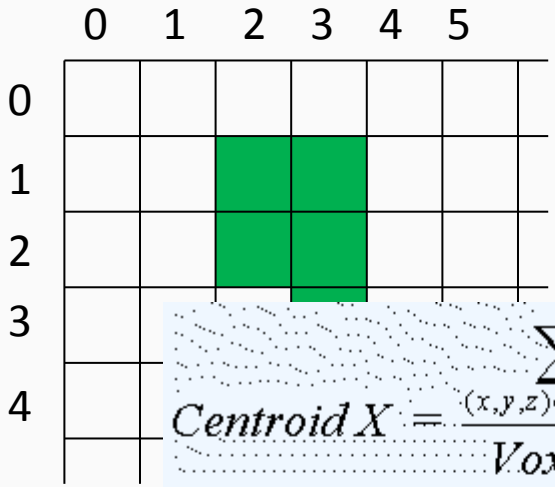
Wikipedia

2 Locating the centroid
- 2.1 Plumb line method
- 2.2 Balancing Method
- 2.3 Of a finite set of points
- 2.4 By geometric decomposition
- 2.5 By integral formula

Of a finite set of points

The centroid of a finite set of k points $\mathbf{x}_1, \mathbf{x}_2, \ldots, \mathbf{x}_k$ in $\mathbb{R}^n$ is

$$\mathbf{C} = \frac{\mathbf{x}_1 + \mathbf{x}_2 + \cdots + \mathbf{x}_k}{k}$$



$$Centroid\,X = \frac{\sum_{(x,y,z)\in Object} x}{Voxels}$$

Centroid Y — The Y coordinate value (in pixels) dividing by the total voxels.

$$Centroid\,Y = \frac{\sum_{(x,y,z)\in Object} y}{Voxels}$$

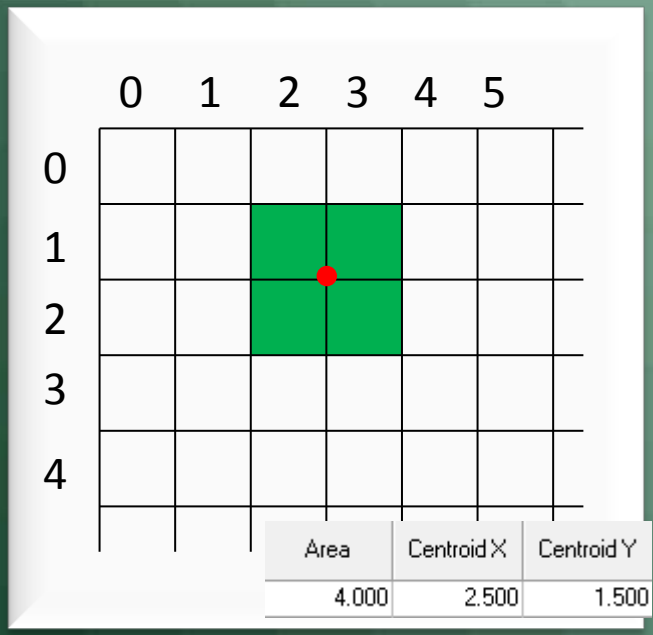
2+2+3+3+3
8

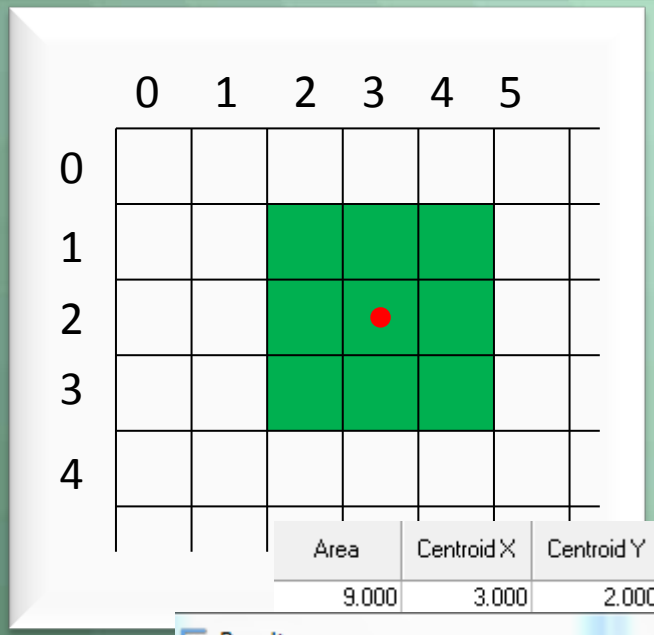
Area
8.0

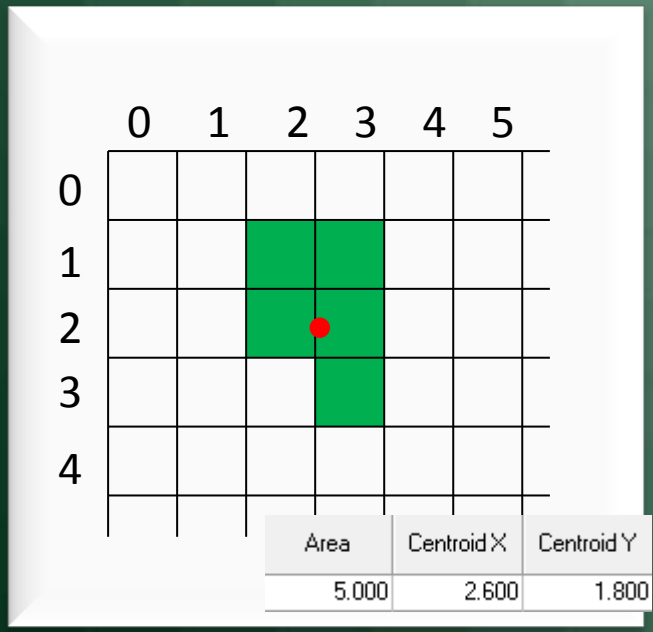
Centroid Z — The Z plane number of the object then dividing by the total voxels.

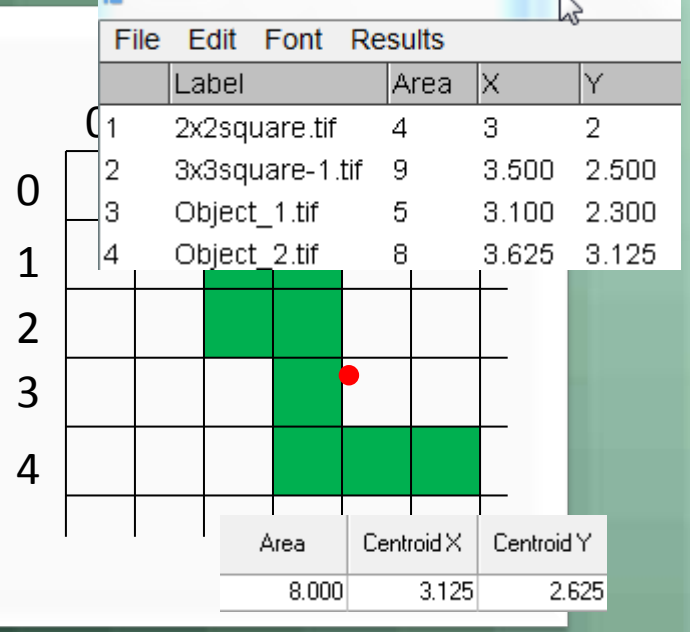
$$Centroid\,Z = \frac{\sum_{(x,y,z)\in Object} z}{Voxels}$$

Position







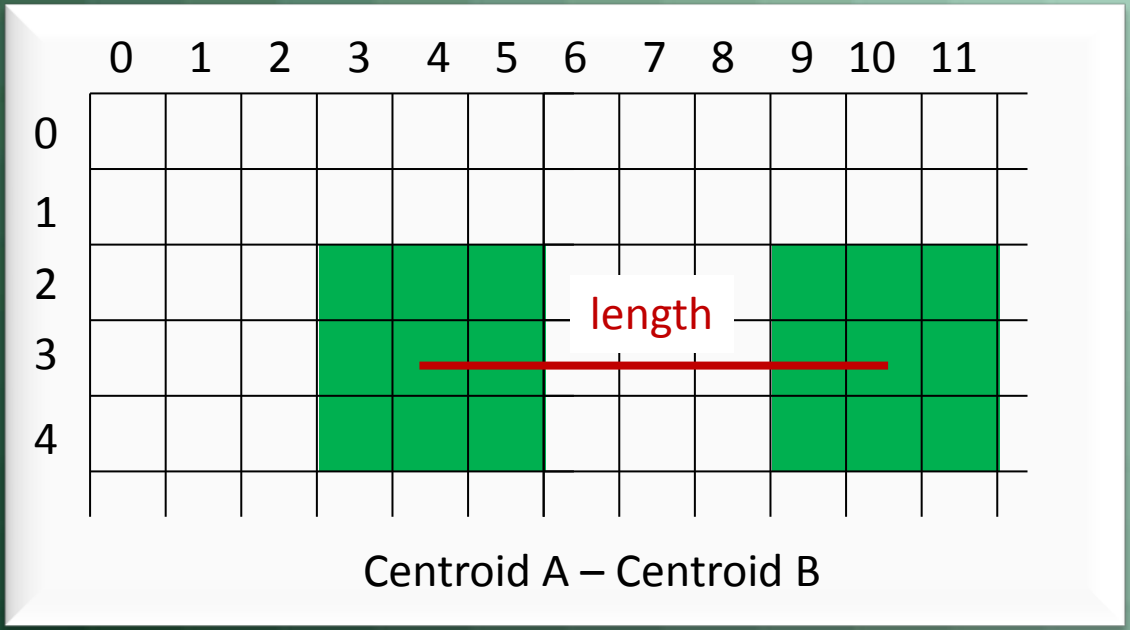


Results

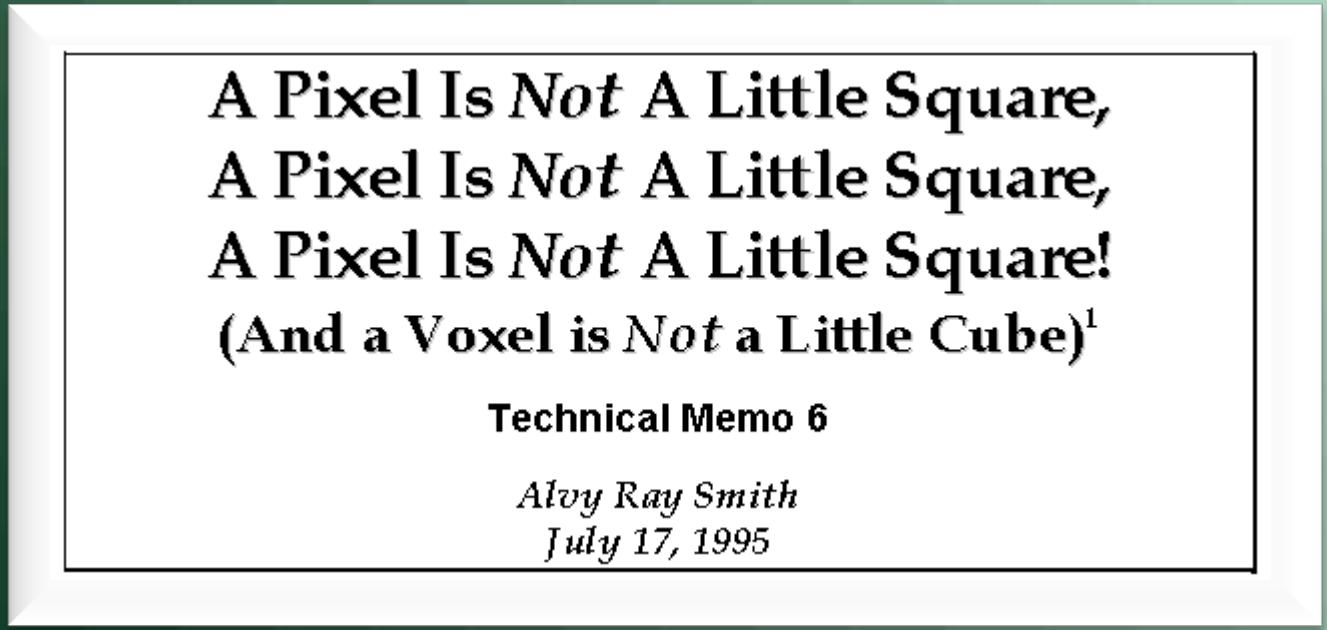
File Edit Font Results

	Label	Area	X	Y
1	2x2square.tif	4	3	2
2	3x3square-1.tif	9	3.500	2.500
3	Object_1.tif	5	3.100	2.300
4	Object_2.tif	8	3.625	3.125

Positon – relative (distance)



The pixel – square problem



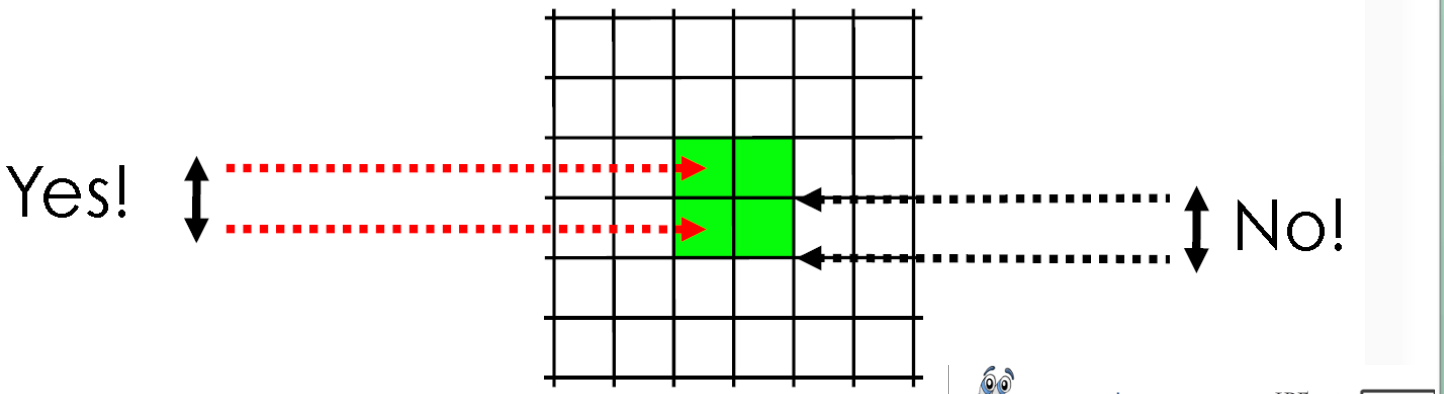
A Pixel Is *Not* A Little Square,
A Pixel Is *Not* A Little Square,
A Pixel Is *Not* A Little Square!
(And a Voxel is *Not* a Little Cube)[1]

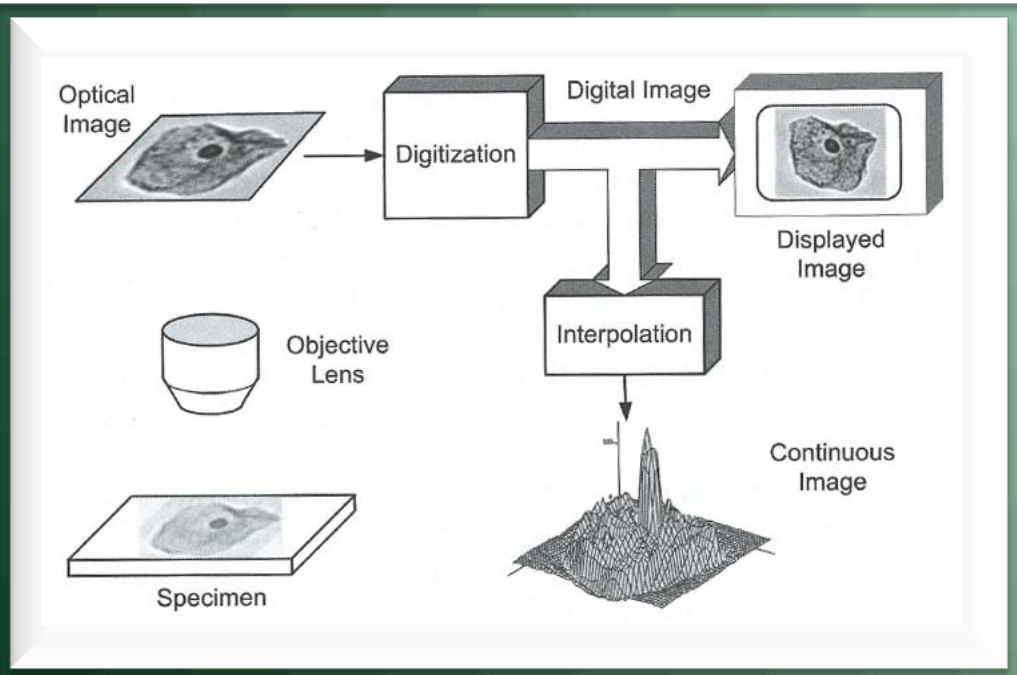
Technical Memo 6

Alvy Ray Smith
July 17, 1995

http://alvyray.com/Memos/CG/Microsoft/6_pixel.pdf

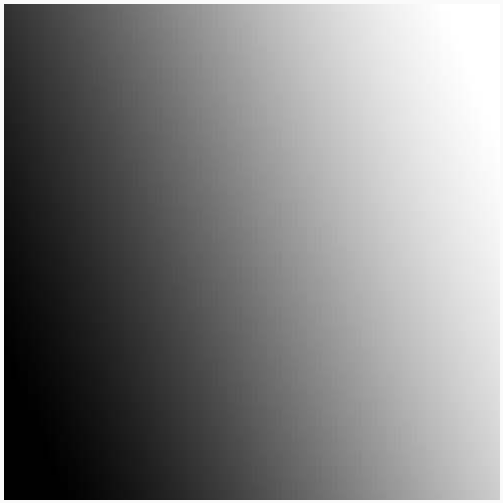
The pixel – square problem



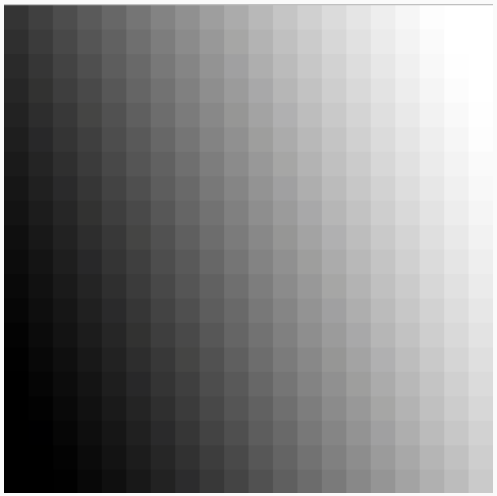


The pixel – square problem

Continuous Light



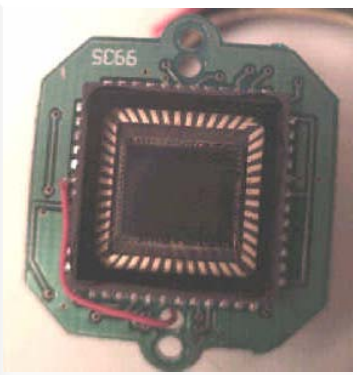
Spatial Sampling/Temporal Sampling

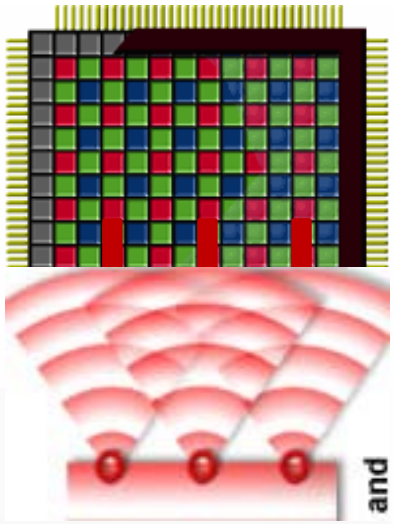


Digitalization

52	64	77	90	103	…	…	…	238	246	253	255	255
47	59	72	85	97	…	…	…	235	243	250	255	255
…	…	…	…	…	…	…	…	…	…	…	…	….
…	…	…	…	…	…	…	…	…	…	…	…	….
…	…	…	…	…	…	…	…	…	…	…	…	….
…	…	…	…	…	…	…	…	…	…	…	…	….
…	…	…	…	…	…	…	…	…	…	…	…	….
…	…	…	…	…	…	…	…	…	…	…	…	….
0	0	3	11	19	…	…	…	155	168	181	193	206
0	0	1	8	15	…	…	…	149	163	176	188	202

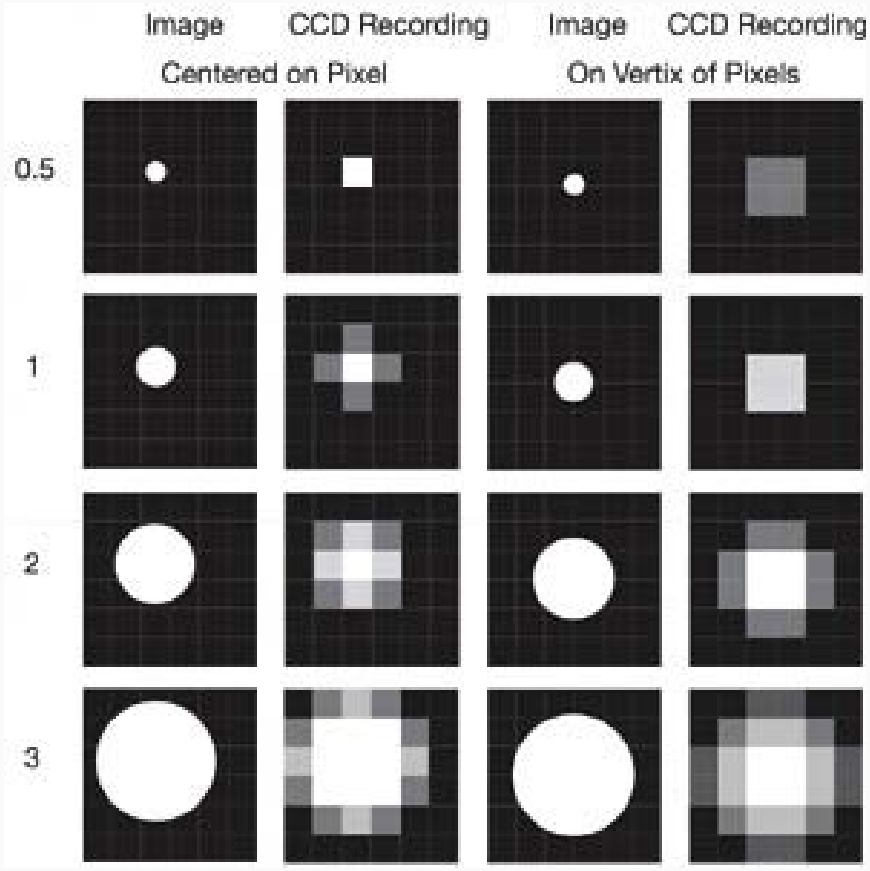
The pixel – square problem



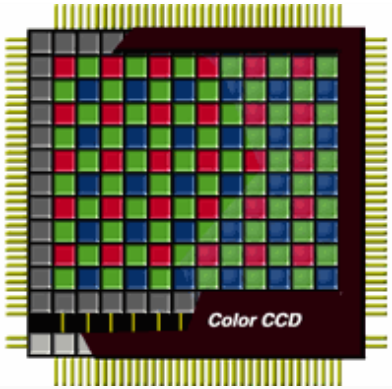


“Microscope Problem”

“Localization Problem”



The pixel – square problem



Read Out

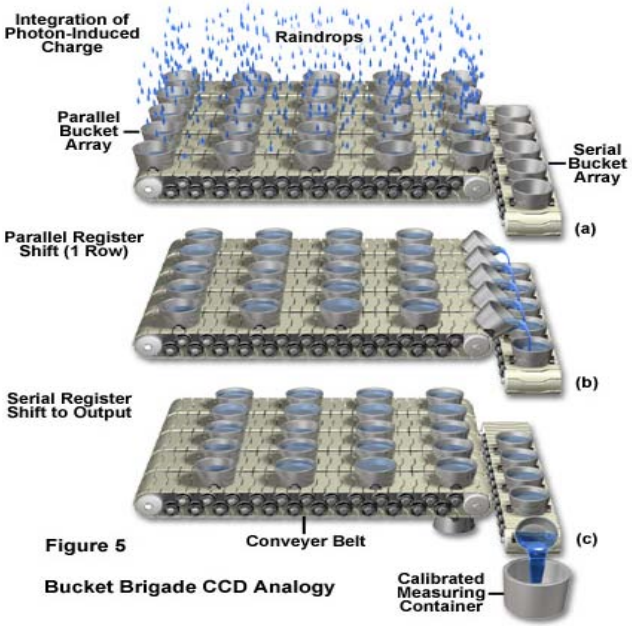
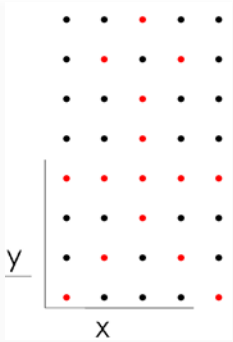
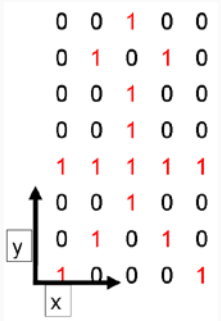


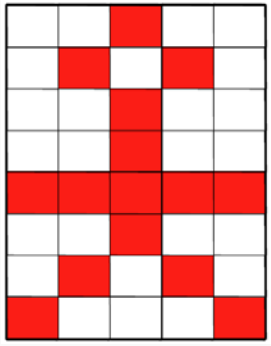
Figure 5
Bucket Brigade CCD Analogy

"Detector Problem"





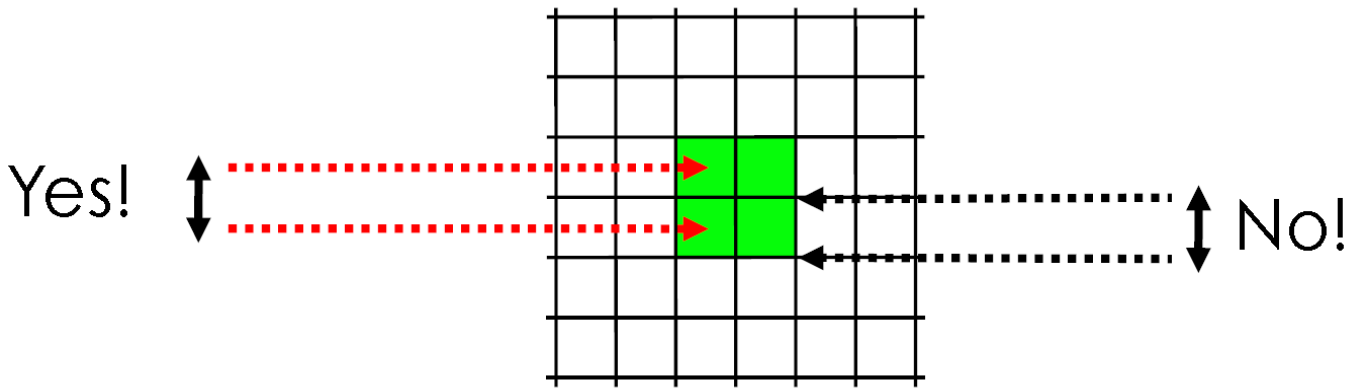
This is a simple way to show this.



The pixel – square problem

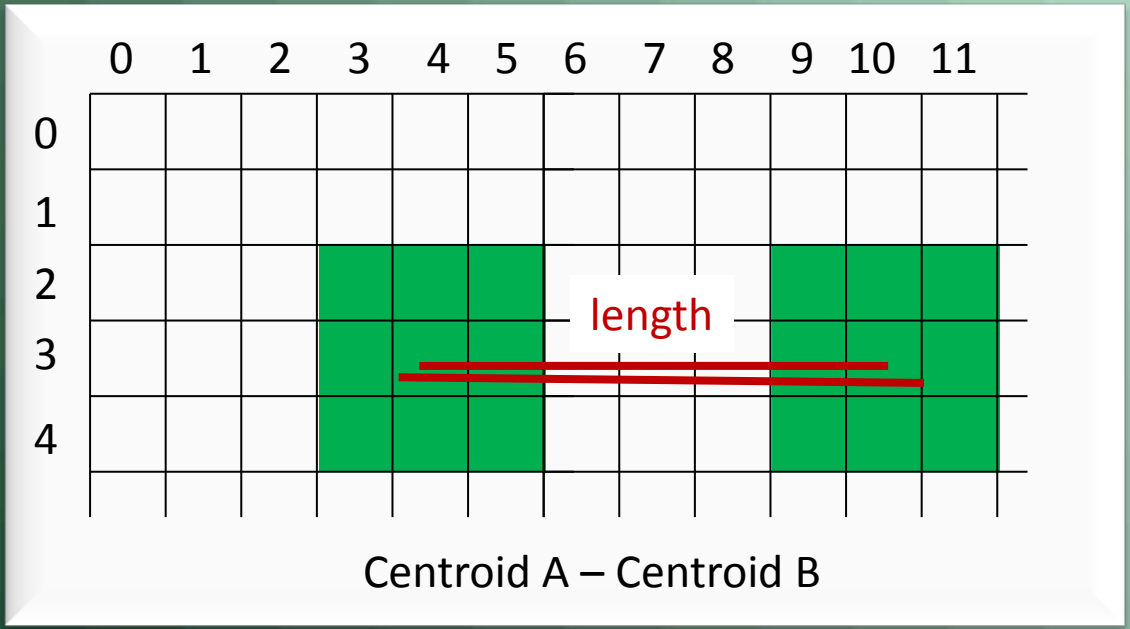
A pixel is a sample of "intensity" from a POINT in space

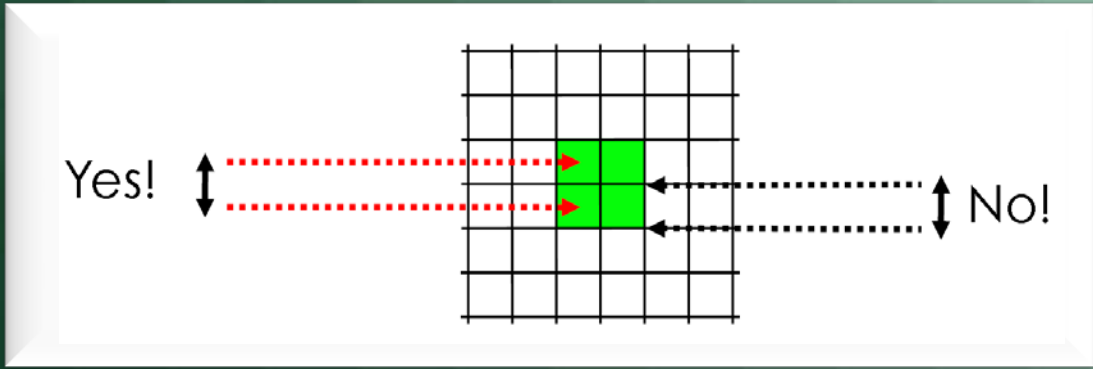
"pixel size" is pixel spacing distance, not the imaginary pixel edge length!



A pixel is not a little square!

Positon – relative (distance)





Position - Tools

Metamorph

- Measure > Measure Pixel
- Measure > Integrated Morphological Analysis
- 4D Viewer > Measure > Measure Objects

Fiji

- Analyze > Measure
- Analyze > Analyze Particles
- View5D > Marker

Absolute Position

Centroid
The center point of the selection. This is the average of the x and y coordinates of all of the pixels in the selection.

Center of Mass
This is the brightness-weighted average of the x and y coordinates all pixels in the selection.

Metamorph

- Measure > Integrated Morphological Analysis
- Measure > Region Measurements
- Apps > Measure XYZ Distance
- 4D Viewer > Measure > Measure Distances
  (centroid distance, shortest distance)

Fiji

- Analyze > Measure
- Analyze > Analyze Particles
- View5D > Marker

Relative Position

What to measure?

What?

- Position
- Distance
- Number
- Extend
- Shape
- **Intensity**

Counting - Tools

Metamorph

- Measure > Manually count objects
- Measure > Integrated Morphological Analysis (2D)
- 4D Viewer > Measure > Measure Objects

Fiji

- Analyze > Analyze Particles (2D)
- Analyze > 3D Objects counter
- Plugin > Process > Particle Analyser (3D)

What to measure?

What?

- Position
- Distance
- Number
- Extend
- Shape
- **Intensity**

Dimension Measurement Terms

Width

The horizontal dimension of the object.

Height

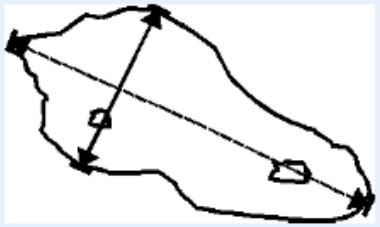
The vertical dimension of the object.

Bounding Rectangle - The smallest rectangle enclosing the selection. Uses the headings ***BX***, ***BY***, ***Width*** and ***Height***, where *BX* and *BY* are the coordinates of the upper left corner of the rectangle.

Length

The span of the longest chord through the object.

Breadth



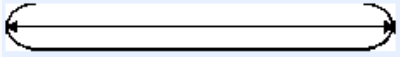
The caliper width of the object, perpendicular to the longest chord. (See also *Length.*)

Fit Ellipse - Fit an ellipse to the selection. Uses the headings ***Major***, ***Minor*** and ***Angle***. *Major* and *Minor* are the primary and seconday axis of the best fitting ellipse. *Angle* is the angle between the primary axis and a line parallel to the x-axis of the image.

Fiber Length

$$\text{Fiber Length} = \frac{1}{4}\left(P + \sqrt{P^2 - 16A}\right)$$

Where P = Perimeter and A = Area

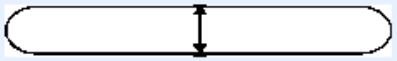


The length of an object, assuming that it is a fiber.

Fiber Breadth

$$\text{Fiber Breadth} = \frac{1}{4}\left(P - \sqrt{P^2 - 16A}\right)$$

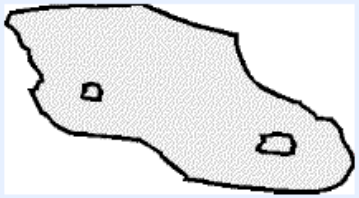
Where P = Perimeter and A = Area



The width of an object, assuming that it is a fiber.

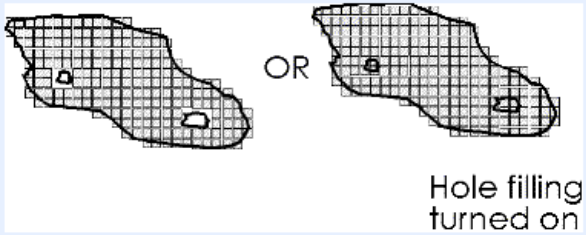
Area Measurement Terms

Total Area



The area of the entire object, including any holes present, regardless of hole-filling.

Pixel Area



The number of *pixels* in the object.

(Pixels inside holes are not included unless hole-filling is enabled in the Measure Objects Preferences dialog box.)

Hole Area

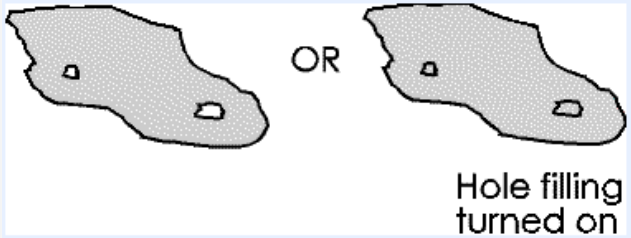
The area of the holes in the object.

If hole-filling has been enabled in the Measure Objects Preferences dialog box, this area should be equal to 0.

Area - Area of selection in square pixels. Area is in calibrated units, such as square millimeters, if *Analyze>Set Scale* was used to spatially calibrate the image.

Area Fraction - The percentage of pixels in the image or selection that have been highlighted in red using *Image>Adjust>Threshold*. For non-thresholded images, the percentage of non-zero pixels.

Area



The area of the object in *calibrated units*.

(The area inside holes is not included unless hole-filling is enabled in the Measure Objects Preferences dialog box.)

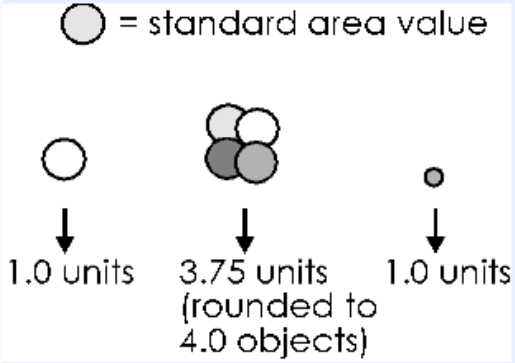
Relative Hole Area

$$\text{Relative Hole Area} = \frac{\text{Hole Area}}{\text{Total Area}}$$

The ratio of the *hole area* to the *total area* of the object.

A relative hole area of 0 indicates that the object has no holes, whereas a relative hole area near 1 indicates that the object consists mostly of holes.

Standard Area Count

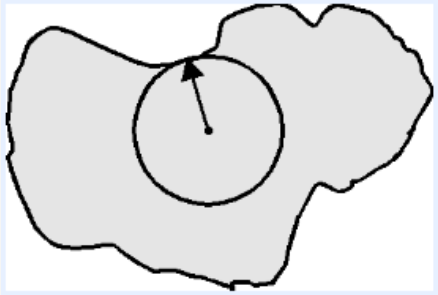


The number of times larger that the object is than the value defined as the *standard area*.

If an object has a standard area count less than 1.0, it will count as a single object.

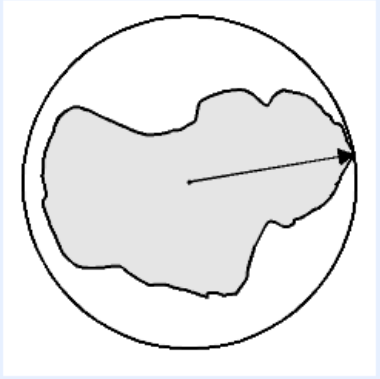
Circular Measurement Terms

Inner Radius



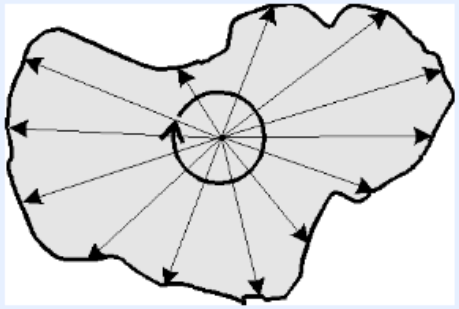
The distance from the centroid to the nearest point along the object's edge.

Outer Radius



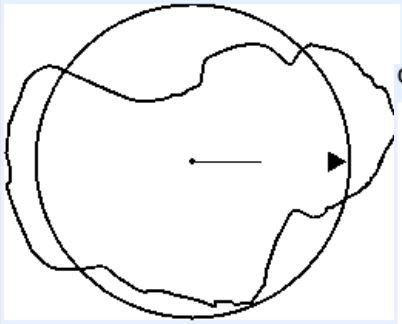
The distance from the centroid to the farthest point along the object's edge.

Mean Radius



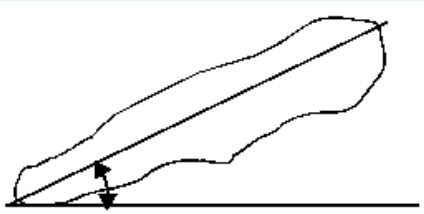
The average distance from the centroid to all points along the object's edge.

Equivalent Radius



The radius of a circle that would contain an that of the object.

Orientation



The angle between (1) the longest chord throug and (2) the horizontal axis. The orientation will 90 to +90 degrees.

Shape Factor

$$\text{Shape Factor} = \frac{4\pi A}{P^2}$$

Where P = Perimeter and A = Area

A value from 0 to 1 representing how closely the object represents a circle.
A value near 0 indicates a flattened object, whereas a value of 1.0 indicates a perfect circle.

Elliptical Form Factor

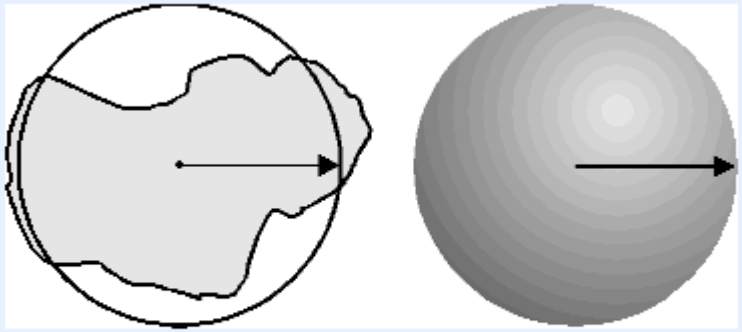
$$\text{Elliptical Form Factor} = \frac{\text{Length}}{\text{Breadth}}$$

The ratio of the object's breadth to its length.

Shape Descriptors **(previously *Circularity*) :**
Circ. **(circularity):** 4π*area/perimeter^2. A value of 1.0 indicates a perfect circle. As the value approaches 0.0, it indicates an increasingly elongated shape. Values may not be valid for very small particles.
AR **(aspect ratio):** major_axis/minor_axis.
Round **(roundness):** 4*area/(π*major_axis^2), or the inverse of the aspect ratio.
Solidity: area/convex area.
Feret's Diameter **-** The longest distance between any two points along the selection boundary, also known as maximum caliper. Uses the *Feret* heading. The angle (0-180 degrees) of the Feret's diameter is displayed under *FeretAngle*, as well as the minimum caliper diameter (*MinFeret*). The starting coordinates of the Feret's diameter (*FeretX* and *FeretY*) are also displayed.

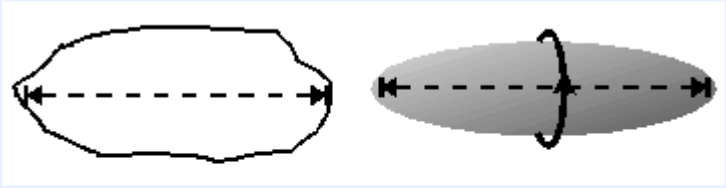
Circular Measurement Terms

Equivalent Sphere Volume



The volume of a sphere that would have an equatorial cross-sectional area equal to that of the object.

Equivalent Prolate Volume



The volume of a prolate spheroid (cigar-shaped object) with a major axis matching that of the object.

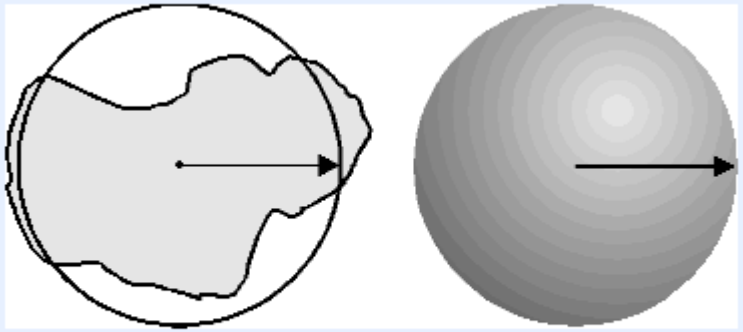
A prolate spheroid is produced by revolving an ellipse around its major axis.

Equivalent Oblate Volume

The volume of an oblate spheroid (squashed sphere or disk) with a minor axis matching that of the object.

An oblate spheroid is produced by revolving an ellipse around its minor axis.

Equivalent Sphere Surface Area



The surface area of a sphere with a cross-sectional area equal to that of the object.

Measuring - Tools

Metamorph

- Measure > Region Measurements
- Measure > Integrated Morphological Analysis (2D)
- 4D Viewer > Measure > Measure Objects

Fiji

- Analyze > Measure
- Analyze > Analyze Particles (2D)
- Analyze > 3D Objects counter
- Plugin > Process > Particle Analyser (3D)
- View5D > Marker

What to measure?

What?

- Position
- Distance
- Number
- Extend
- Shape
- **Intensity**

Where to measure?

Where?

- in the whole image

-at a specific point

- in a region
    - along a line (linescan)

- within a threshold

- in an object

Measuring - Tools

Metamorph

- Measure > Show Region Stats
- Measure > Region Measurements
- Measure > Measure Pixel
- Measure > Integrated Morphological Analysis (2D)
- Measure > Linescan
- 4D Viewer > Measure > Measure Objects

Fiji

- Analyze > Measure
- Analyze > Analyze Particles (2D)
- Analyze > 3D Objects counter
- Plugin > Process > Particle Analyser (3D)
- View5D
- Analyze > Plot Profile
- Plugins > Analyze > Dynamic ROI Profiler