LES ATELIERS DU CIF

CELLULAR IMAGING FACILITY

MANIPULATION D'IMAGES

Yannick KREMPP

IMAGE FILE HANDLING

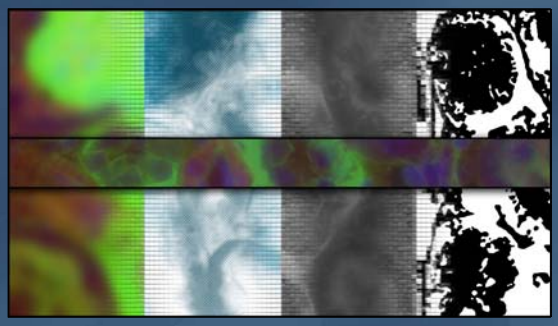

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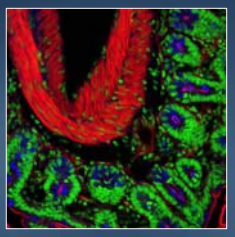
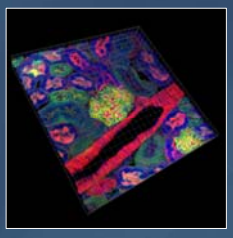
Image handling / processing I

Digital image coding
Sampling
Resolution and definition
Common formats
Image compression
Channels
Common image processing software





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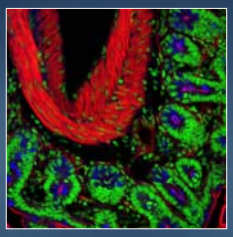

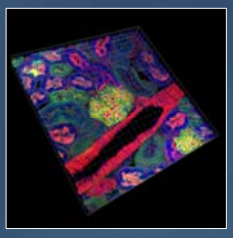

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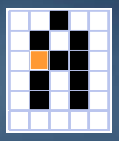
Coding of digital images

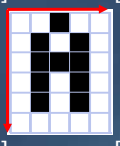
2D Image

An image is constituted of a ensemble of points called **pixels** (abbreviation of ***PICture ELement***)



The pixel represents the smallest constitutive element of a digital image. All these pixels are contained in a matrix (array) of two dimensions that constitutes the image:

[0,0] [5,0]



[0,6] [5,6]

Image of 5x6 pixels

As the screen is scanned from left to right, and from up to down, one generally gives the coordinate [0,0] to the upper left pixel.

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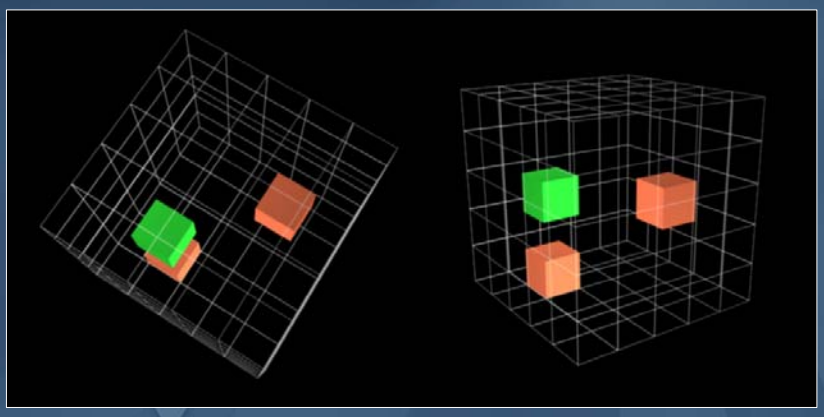
Coding of digital images

3D Image

In a similar way, the 3D image is constituted of an ensemble of points called **voxels** (abbreviation of ***VOlume ELement***)



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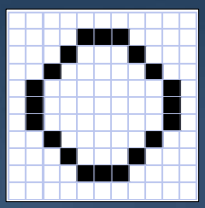
Coding of digital images

Bitmap or vector image?

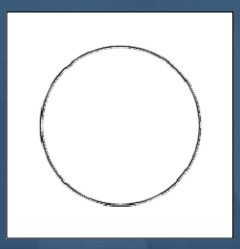


Bitmap images (also called *raster images*) :
They are pixel images, i.e. an ensemble of points (pixels) contained in a table (array), each of these points possessing one or more values describing its color.

With this type of images, it is possible to represent complex scenes (photographs), but with a quality loss when the image it too much magnified.

Vector images: they are representations of geometical entities (circle, rectangle, segment). They are represented by mathematical formulas and equations (sinusoids, beziers, etc.).

This type of images allow representing simple scenes and make them undego transformations (magnification, rotation, etc.) without altering their quality.



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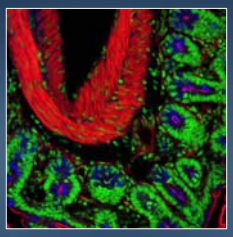

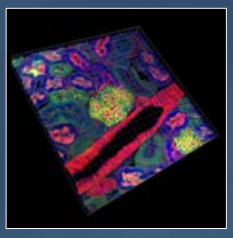

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Sampling

-An image is represented by a 2-dimensional array where each element is a pixel

-The value stored in each coordinated is coded using a certain number of bits that determine the color or the intensity of a pixel. This is called the **bit depth**.

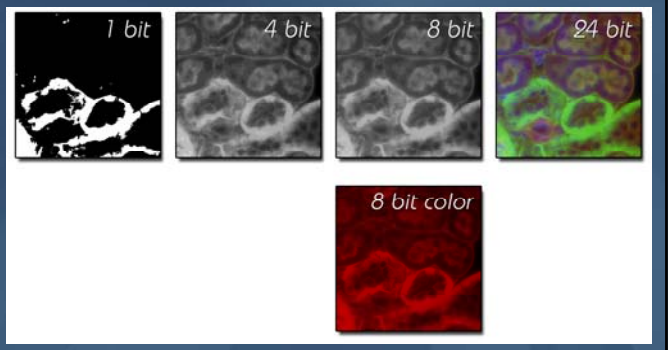
1 bit = bitmap = 2 levels (black and white)

2 bits = 4 levels of grey

4 bits = 16 levels of grey

8 bits = 256 levels of grey or of a color

24 bits = true color = RGB = 3x8 bits colors = 16 millions colors



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Sampling

The weight of an image in kilobytes (Kb) is directly linked with the sampling and bit depth of an image.

(Numbre of pixels in X) x (number of pixels in Y) x (number of bits) / 8 /1024 = image weight in kbytes

to get the image definition

to get pixel weight in bytes

to get weight in kilo-bytes

Examples of weight for non-compressed images

Image definition	Black and white (1 bit)	256 greys (8 bits)	65000 colors (16 bits)	True color (24 bits)
320x200	7.8 Ko	62.5 Ko	125 Ko	187.5 Ko
640x480	37.5 Ko	300 Ko	600 Ko	900 Ko
800x600	58.6 Ko	468.7 Ko	937.5 Ko	1.4 Mo
1024x768	96 Ko	768 Ko	1.5 Mo	2.3 Mo

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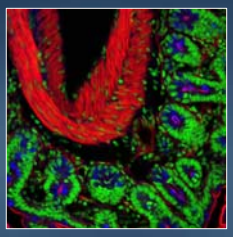

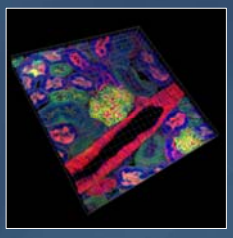

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Image Resolution and Definition

Definition: it is the size of the image in pixels. An image with 640 pixels in width and 480 pixels in height will have a definition of 640 by 480 pixels, written *640x480*.

Resolution: determined by the number of pionts per unit of surface, expressed in point per inch (**DPI** for *Dots Per Inch*); one inch being 2.54 cm.

The resolution allows giving the relationship between the number of pixels of an image and its real size on a physical support.

A few practical considerations:

Computer screen has a maximum resolution of 72 dpi.
A paper print of *average* resolution is 150 dpi.
A paper print of *good* resolution is higher than 300 dpi (required by copy editors).

Practical case: to make the cover of Science, you need to fill a sheet of 26.6 x 20.9 cm, i.e. 10.47 x 8.23 inches, in 300 dpi. The image definition has therefore to be at least 3141 x 2469 pixels …

Image Resolution and Definition

A few practical considerations (continued)

Flat-bed scanner is capable of generating images with very high resolutions (more than 10,000 dpi interpolated). This resolution is only necessary for making magnifications from negatives or slides.

In summary:

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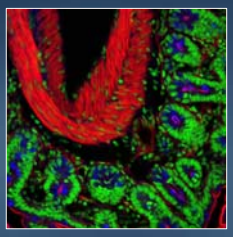

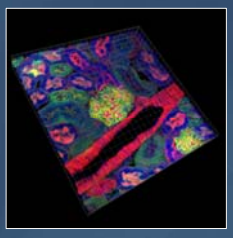

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Common Formats

General imaging

TIFF :	very usual format used by numerous image processing software packages. It can use the LZW compression algorithm (loss-less) and also the Packbits / CCITT G3&4 / RLE / JPEG / LZW / UIT-T .
JPEG :	format used mainly for images used for visualization. Very efficient compression but with loss of information. Uses exclusively the mode of compression of its own name.
BMP :	Windows bitmap. No compression or RLE.
GIF :	Used mainly for websites. 256 colors max, possibility of animations.
PNG :	Portable Network Graphics, used mainly for websites, allows adding an transparency channel – alpha.
PSD :	Photoshop proprietary format. Works with layers.
AI :	Illustrator format.
EPS:	Encapsulated Postscript. Format generated by photoshop for printing purpose.
PDF:	format mainly used for documents but can also contain images. Advantage of being read by about any system with excellent printing quality.

Common Formats

Microscopy imaging

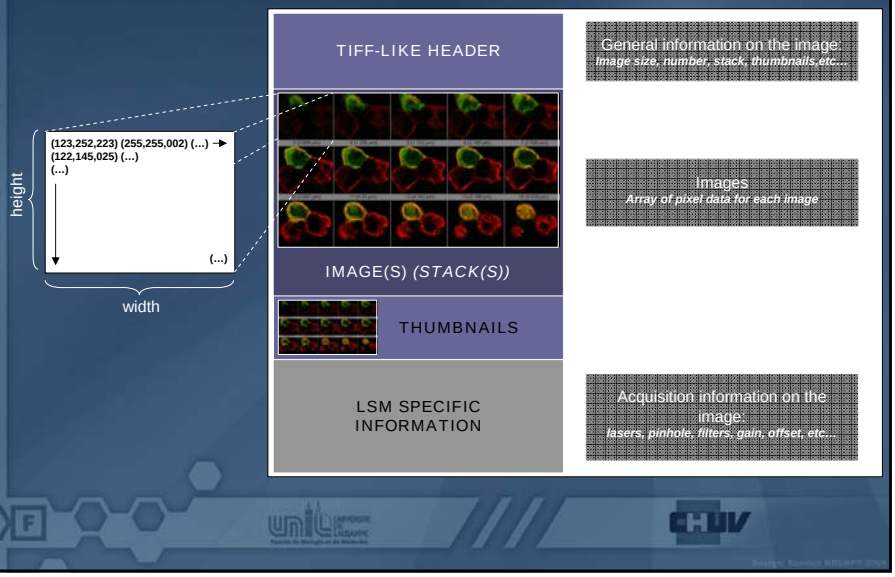
LSM :	Zeiss proprietary format used for their confocal microscopes LSM 510, 510 Meta, Live and Pascal.
ZVI :	Zeiss proprietary format used for the software Axiovision.
LIF:	Leica proprietary file format (new).
PICT :	Biorad format. (same file extension used by Apple computers...).
IMS/IMX:	Format used by Bitplane Imaris.
OME XML:	Open Microscopy Environment.

These formats contain both images and metadata (information on calibration, experimental annotations, etc.) in the same file. The conversion of these formats into another one (e.g. TIFF) causes metadata to disappear.

Other microscope companies propose storing images in classical image formats, with metadata stored separately in text files.

Common Formats

Example: the LSM format



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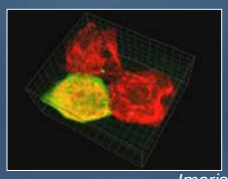
Common Formats

3D Imaging

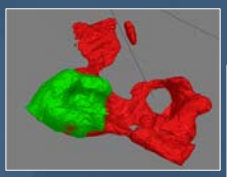
The world of 3D imaging is quite complex but just like video, it begins to be found routinely in the toolbox of researchers.

Several file formats also exist:

-3DS	*Discreet 3D Studio*
-MAX	*Discreet 3D Studio Max*
-OBJ	*Alias Wavefront, Bryce*
-VRML/WRL	*Virtual Reality Modeling Language*
-IV	*Autodesk Inventor*
-DXF	*Autocad ASCII Files*
-LW	*Lightwave*
-PDB/MOL	*Protein Data Bank*
-XSI	*Softimage XSI*



Imaris



3D Studio Max

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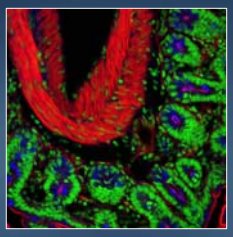

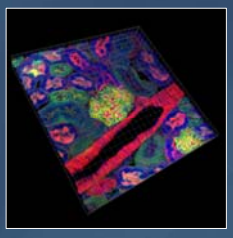

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Image Compression

One can distinguish two categories of compressions:

-The compression without information loss (« *lossless* »), example: LZW which can reach a maximum compression factor of 2:1, used by the TIFF format.

- The compression with information loss *(« lossy »),* example: JPEG (Joint Photographic Experts Group), which can reach compression factors of a maximum of 10:1 or even 20:1 in certain cases.

It exploits the ability of our visual system to better distinguish variatinons in luminosity and much less variations in colors.

This type of compression is useful for visualization and edition, but should be avoided for quantification.

Compression JPEG under Photoshop.

NB: each software implements the JPEG algorithm differently.

Source: http://www.photo.net/learn/jpeg/

Image Compression

Original **Compressed** **Difference image**

Other illustration of information loss during **JPEG** compression .

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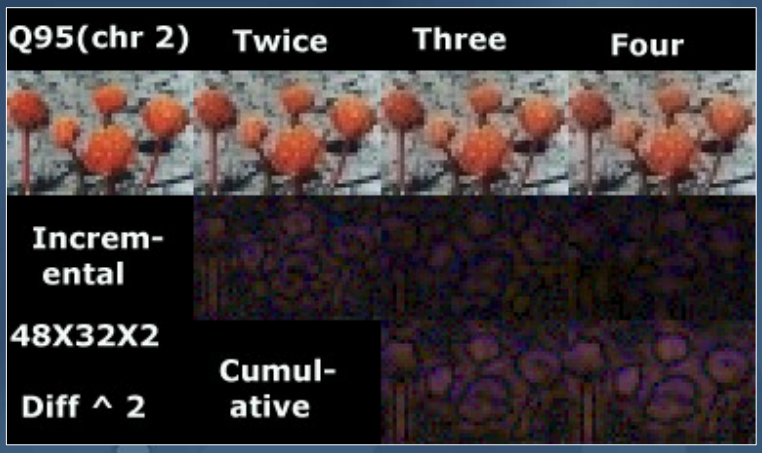
Image Compression

Furthermore, a succession of edition – saving cycles with the same compression factor also degrades images: example with a simple down and left shift with subsequent saving of the image



Source: http://www.photo.net/learn/jpeg/

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Image Compression

A few words on video:

More and more digital video is used either for videomicroscopy (time-lapse) or 3D representations.

However, video a nothing else than a sequence of images displayed very rapidly. It can therefore also be compressed.

The different types of coding are here called CODEC (compression/decompression):

- MJPEG (professional video studios)
- MPEG 1 (VCD), 2 (DVD), 4 (web, portables), 7, 21
- MOV (Quicktime, 3viX)
- AVI (Intel Indeo)
- AVI (CinePak)
- AVI (DivX, XviD) equivalent of MP3 for video.
- MKV (Matroska Video) container DivX+son+texte

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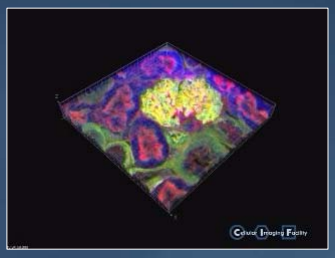
Image Compression

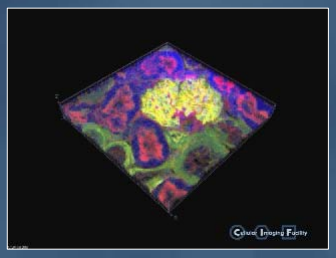
Examples of video compression:

3D Reconstruction using Imaris

Original file size (640x480, non compressed) : 439 Mb



Video (320x240) compressed with Intel Indeo 5.10 codec
File size: 4,19 Mo



Video (320x240) compressed with CinePak codec by Radius
File size: 4,42 Mo

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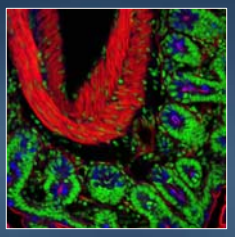

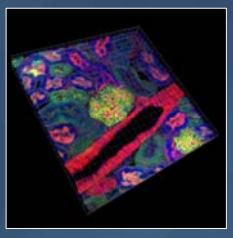

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Image channels

A color image may be split into different channels. The number of channels and the scale of values used depends on the type of coding chosen to represent the color space.

NB: the spectrum of colors that an *output device* is able to represent is called **Gamut** or colorimetric space. Colors that are not contained in the Gamut are said to be *out-of-gamut*. According to the coding used and the gamut, the output device will be or not able to display the image accurately.

Several types of coding

RGB coding	(Red, Green, Blue).
HSL coding	(*Hue, Saturation, Luminance* = *Teinte, Saturation, Luminance* -TSL).
CMYK coding.	(*Cyan, Magenta, Yellow, blacK*)
CIE coding	(*Commission Internationale de l'Énergie*, codage Lab)
YUV coding	(*coding for PAL/SECAM video standards*)
YIQ coding	(*coding for PAL/SECAM video standards*
(…)	
LSM coding	coding used for LSM files of Zeiss confocals. Similar to RGB except that it can have up to 32 channels instead of 3 (red, green, blue).

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Image channels

Example of separation of channels in R, G, B and then in HSL

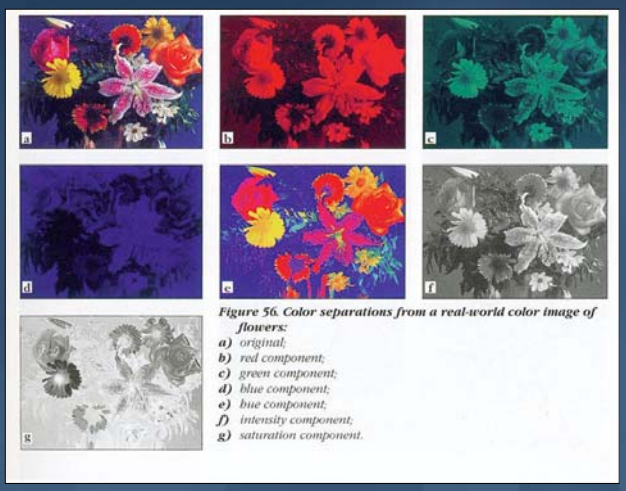

Figure 56. Color separations from a real-world color image of flowers:
a) original;
b) red component;
c) green component;
d) blue component;
e) hue component;
f) intensity component;
g) saturation component.

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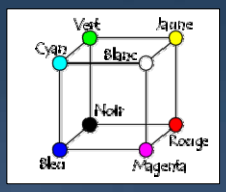
RGB color coding



this color space corresponds to the way colors are generally coded on computers (CRT monitors)

HSL coding

HSL coding, closer to our physiological perception of colors, allows choosing colors more easily than the RGB model.

NB: the human eye is more sensitive to changes of luminance than changes in hue. (It can still distinguish about 2 millions !)

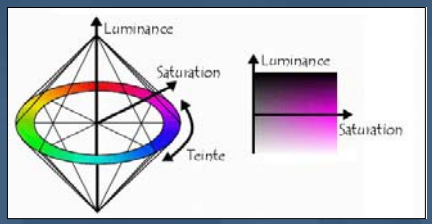


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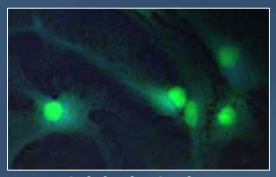
Image channels

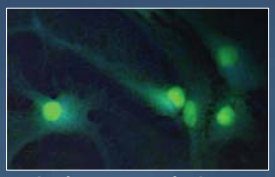
CMYK coding

It is a coding used by copy editors, printers, that correspond to that corresponds to quadrichromy.

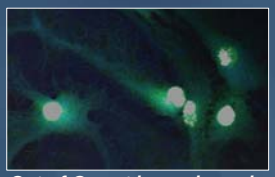
It is important to note that the color space possible in CMYK does not cover the whole range offered by RGB. A digital image acquired in RGB (e.g. in fluroescence) will therefore not look the same once printed, and we will get a faded – watery effect. It is possible to have an idea of the result in Photoshop.



Original RGB image



RGB image seen in CMYK by Photoshop



Out-of-Gamut hues shown by Photoshop (« Gamut warning »)

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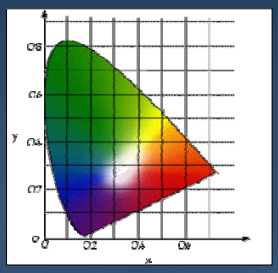
Image channels

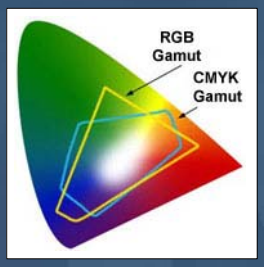
*CIE (La*b*) coding*



Chromaticity diagram showing the Gamut of the human eye (visible spectrum)

Lab coding is based on chromaticity (two components x and y or a and b of "Lab") and the luminance (L).

This model is not intuitive but does guaranty that a color coded this way will be seen correctly regardless of the output device used.



Gamut of RGB coding (e.g. used by a CRT monitor) and of CMYK (e.g. printer) compared with visible spectrum

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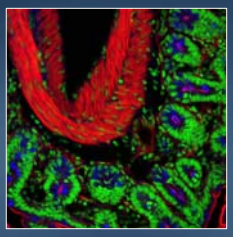
Image handling / processing I

Digital image coding
Sampling
Resolution and definition
Common formats
Image compression
Channels
Common image processing software



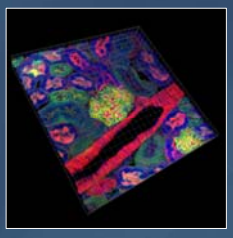
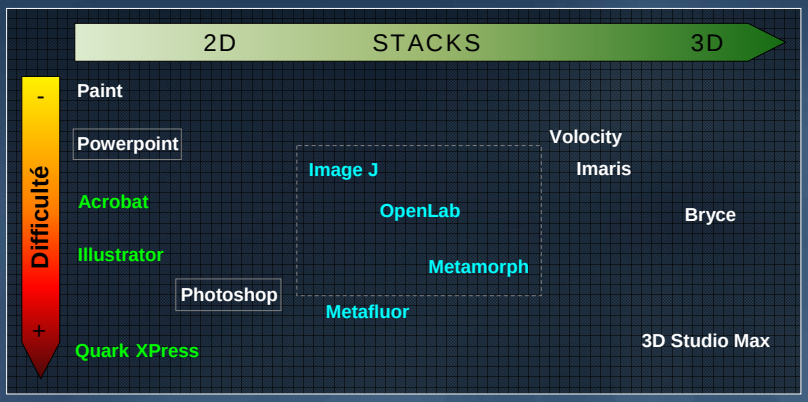


Image handling / processing II

Resizing / Rescaling
Color lookup tables / Inversion
Histogram / Thresholding
Filters / Fourier
Measurements
3D Reconstruction / Deconvolution
Registration / Segmentation

Common software packages



- This listing is not complete.
- It is important to use a given software package within its correct application range (that it was designed for).

Common software packages

A few words on video…

Video also has its "stars". Two examples:

Adobe Premiere: it is the alter ego of Photoshop for video.very useful for video editing and sequences.

VirtualDub: toolbox for video, very convenient to modify attributes of a video file such as compression, framerate, filtering (as in Photoshop). + Freeware !

... and databases

More and more the volume of images stored increase. The size of datasets is today no longer an issue, however, the management of this volume of information is difficult.

Specialized software combining concepts of databases, data mining, and image processing start to appear, such as Image Access.