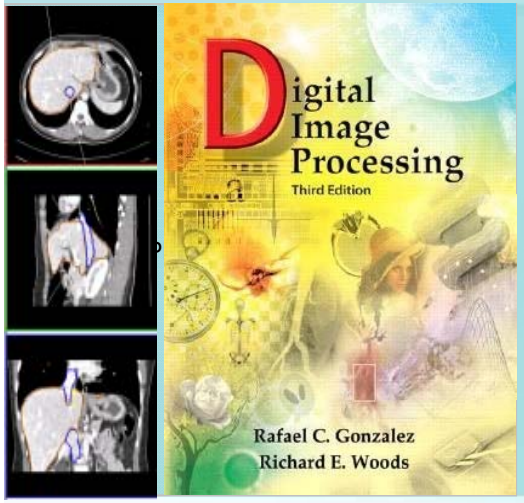
IN THE NAME OF GOD

The most beneficent, the most merciful

Biomedical Engineering Dept.,
Medical Image & Signal Processing Research Center

Isfahan Univ. of Medical Sciences



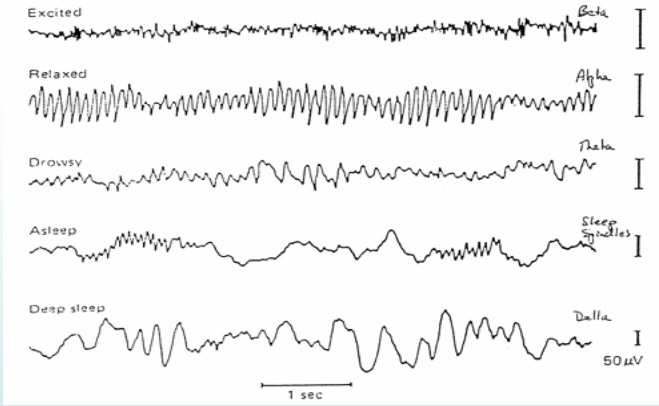

# AN INTRODUCTION TO APPLICATION OF IMAGE PROCESSING IN CELL TRACKING

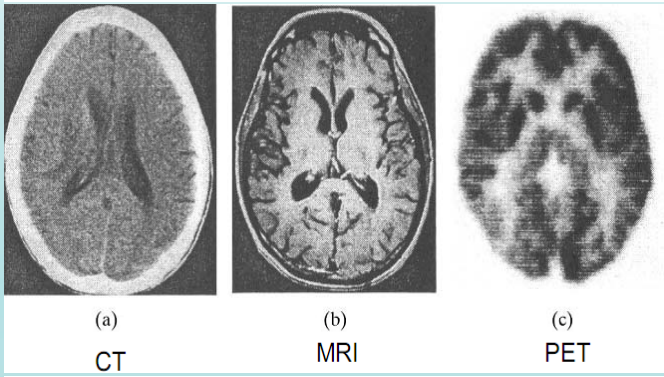
***Presented by:***

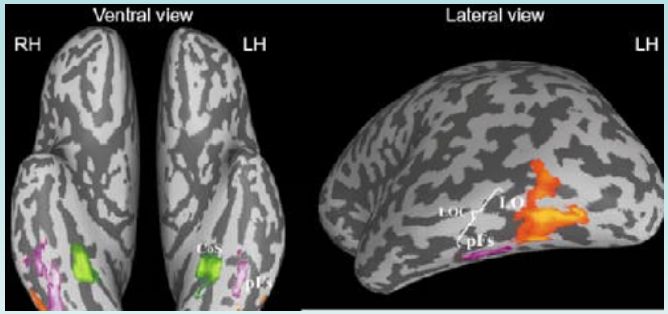
***Dr. Hossein Rabbani***

# Multidimensional Signals

- 1-D Signals (speech, biomedical signals,...)
- 2-D Images (medical images,...)
- 3-D Images (video, medical volume,...)
- 4-D Images (fMRI, cardiac MR,...)

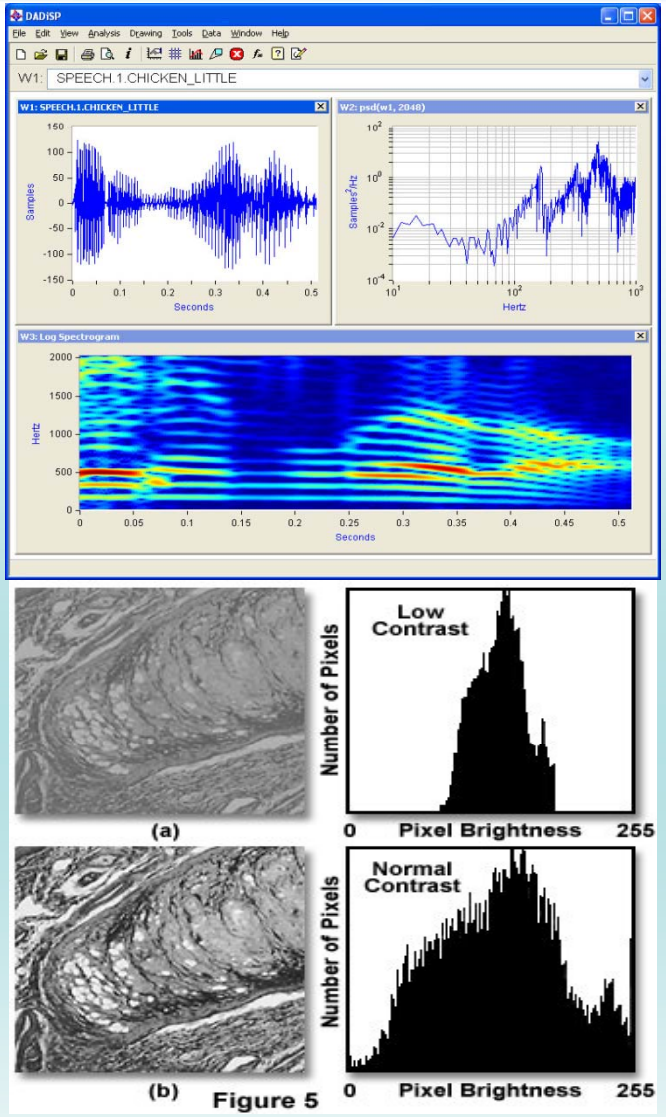


# Why Processing?

- Information
- Examples (preprocessing, feature extraction, classification, medical applications, other applications,....)
- Definitions (Wikipedia, Gonzalez's book, Jain's book, Castleman's book...)



# Why Processing?

## A specific example

- The instrumentation and reconstruction algorithms in PET are highly developed, however, the propagation of noise limits the accuracy of the images obtained using PET. The accuracy can be improved by increasing the injected activity or the scan time, but this is usually not a safe or practical solution. We are investigating the use of wavelet-based denoising of the projection data for increasing the signal-to-noise ratio of the reconstructed image, while have as a goal the negligible loss of resolution.
- The preliminary work suggests that wavelet denoising may be able to increase the signal-to-noise ratio by more than a factor of two.

# What is an image?

***A two dimensional (spatial) array of data, possible time-varying, representing some sensed quantity of interest. We will mostly be concerned with "visual" data.***

- photograph or slide, TV picture, paper document, X-ray image, ultrasound image, geophysical data, etc.

# Application Areas of Image Processing

- **Purpose of image processing**

– Improvement of pictorial information for human interpretation

– Compression of image data for storage and transmission

– Preprocessing to enable object detection, classification, and tracking

- **Typical application areas**

– Television Signal Processing

– Satellite Image Processing

– Medical Image Processing

– Robotics

– Visual Communications

– Law Enforcement

– Etc.

# Application Areas of Image Processing

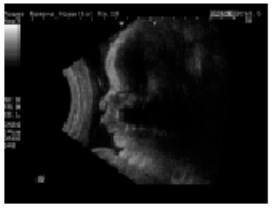
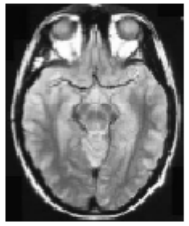
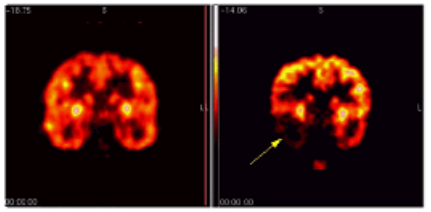
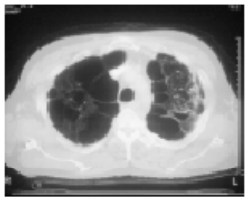
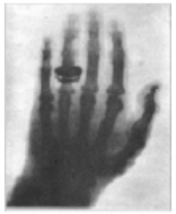
## Television Signal Processing

- Image brightness, contrast, color hue adjustment
- Video compression for efficient delivery and storage
- Conversion among different video formats
  - QVGA<->VGA<->XVGA
  - SDTV<->HDTV
  - NTSC<->PAL

# Application Areas of Image Processing

## Medical Image Processing

- Images are acquired to get information about **Anatomy** and **Physiology** of a patient
- How to reconstruct the image from captured data
- How to process/analyze the image to help diagnosis/treatment?
  - Ultra Sound (US)
  - Magnetic resonance Imaging
  - Positron Emission Tomography (PET)
  - Computer Tomography (CT)
  - XRays

# Application Areas of Image Processing

## Visual Communication

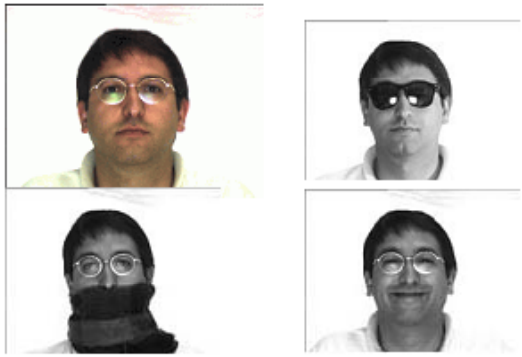
- Videophone
- Tele-conferencing
- Tele-shopping
- How to compress the video to reduce bandwidth/storage requirements
- How to conceal artifacts due to transmission losses?

# Application Areas of Image Processing

## Law Enforcement

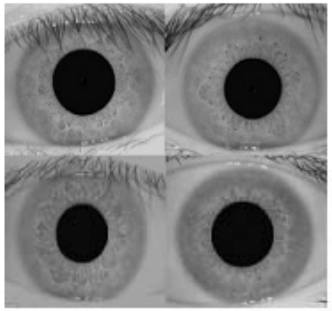
- Biometric identification / verification
  - Fingerprint

  - Face



AR Face Database

  - Iris



  - How to extract features that can be used to differentiate among different images?

# Application Areas of Image Processing

## Robot Control

- Automatic inspection
- Unmanned operations
  - Autonomous Vehicle driving
- How to detect and track the target?

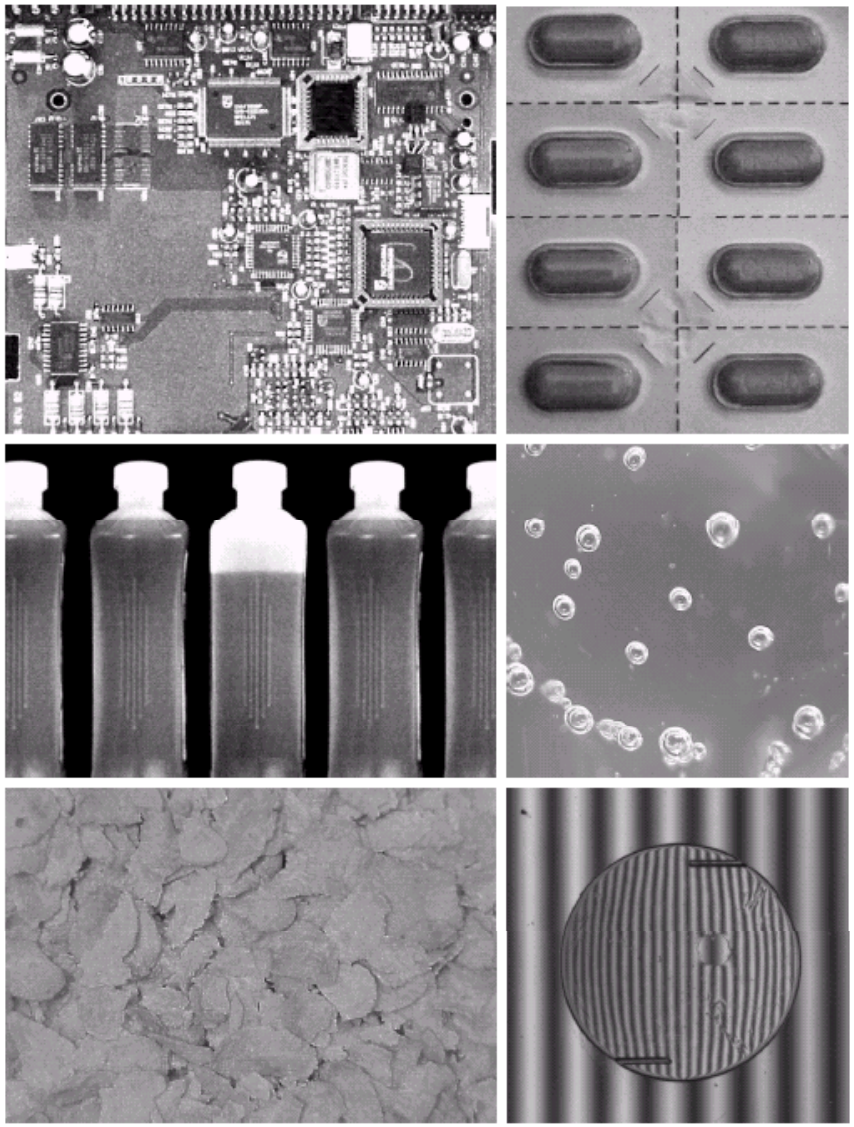
Mars Rover

# Application Areas of Image Processing

## Satellite Image Processing

- Remote sensing
- Climate
- Geology
- Land resource
- Flood monitor
- How to enhance the image to facilitate interpretation?
- How to analyze the image to detect certain phenomena?

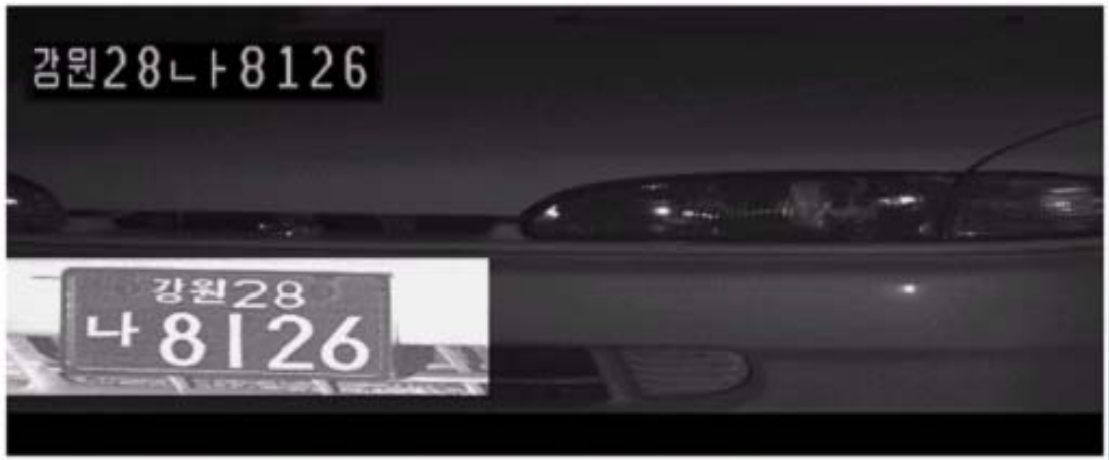
New York (from Landast-5 TM)



a b
c d
e f

**FIGURE 1.14**
Some examples of manufactured goods often checked using digital image processing. (a) A circuit board controller. (b) Packaged pills. (c) Bottles. (d) Bubbles in clear-plastic product. (e) Cereal. (f) Image of intraocular implant. (Fig. (f) courtesy of Mr. Pete Sites, Perceptics Corporation.)

Automatic Reader

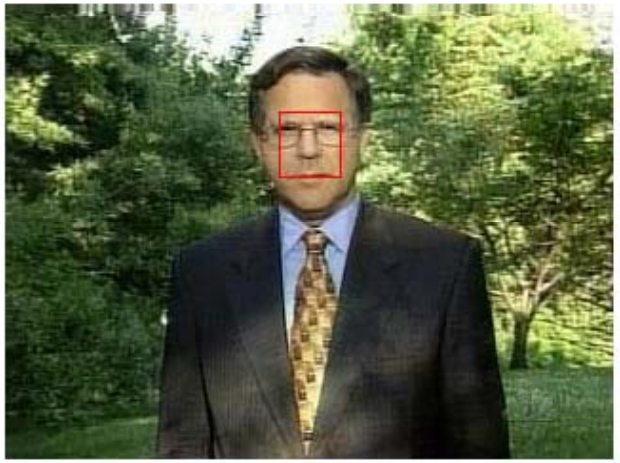



Automatic Reader

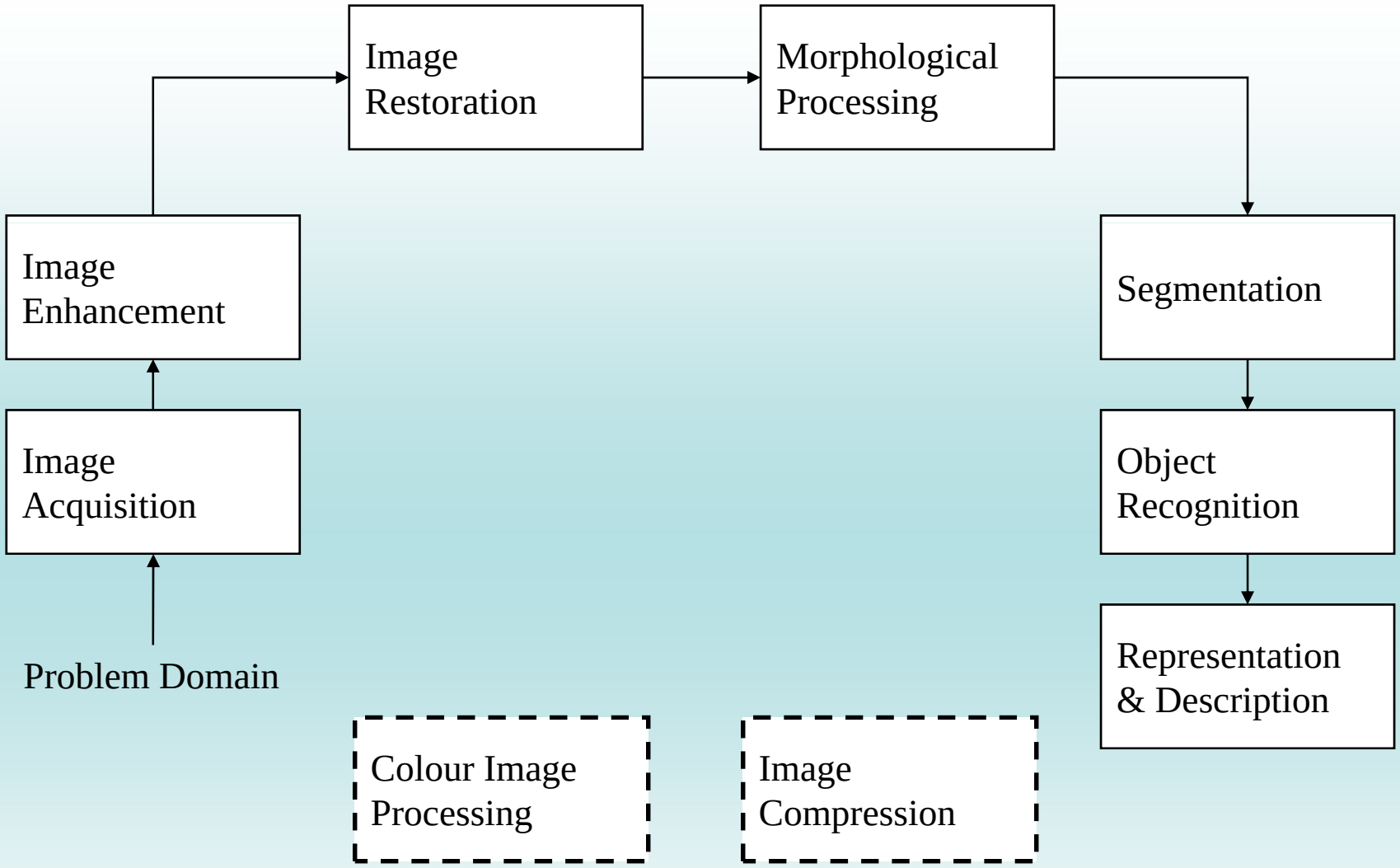
# Basic Image Processing Tools

## Face Detection

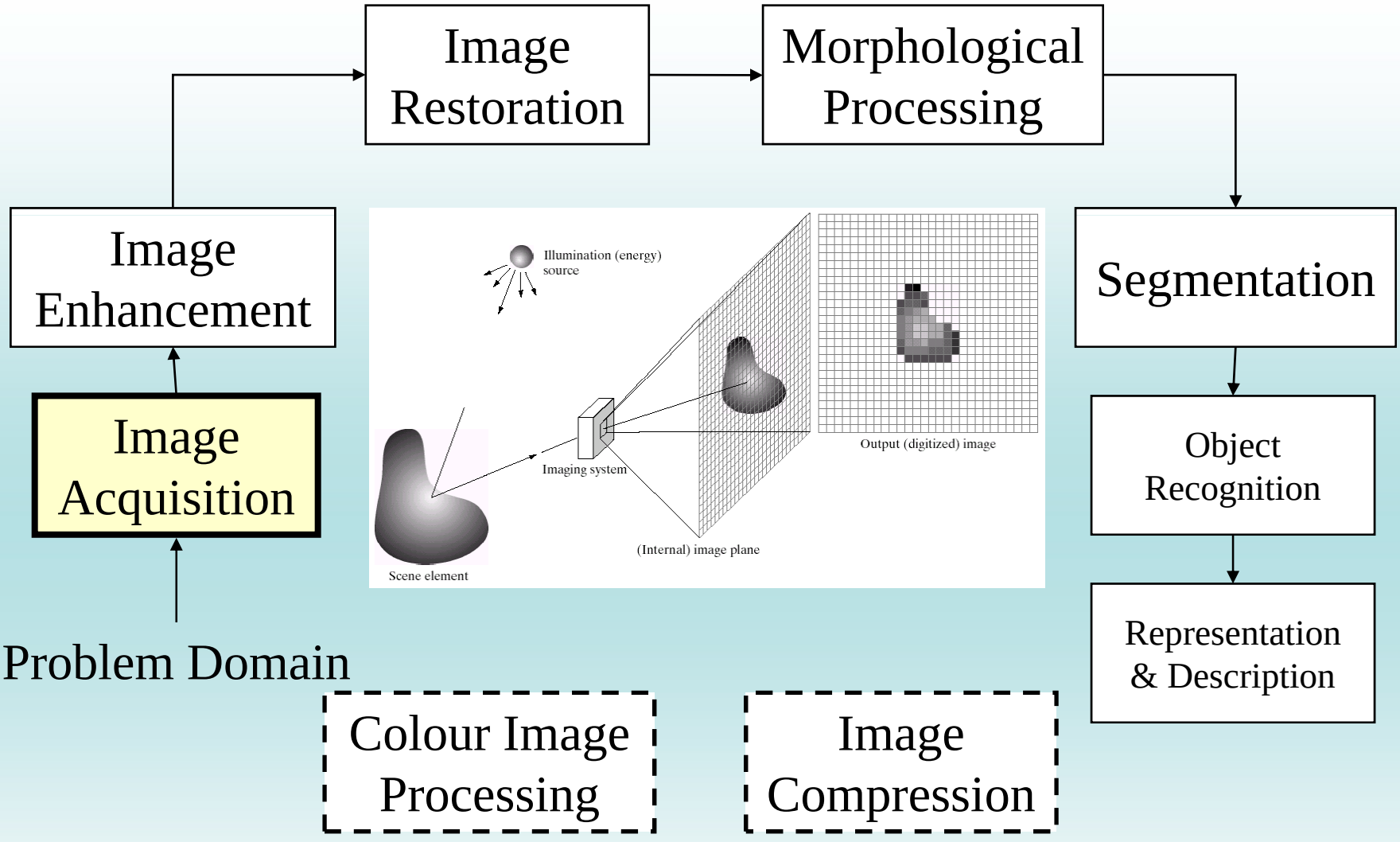
- Face detection in an image or video
- Face tracking in a video

# Key Stages in Digital Image Processing



# Key Stages in Digital Image Processing: Image Acquisition



# Image Formation

- Light source *(λ: wavelength of the source)*
  - $E(x, y, z, \lambda)$: incident light on a point *(x, y, z world coordinates of the point)*
- Each point of the scene has a reflectivity function.
  - $r(x, y, z, \lambda)$: reflectivity function
- Light reflects from a point and the reflected light is captured by an imaging device.
  - $c(x, y, z, \lambda) = E(x, y, z, \lambda) * r(x, y, z, \lambda)$: reflected light.


→ $E(x, y, z, \lambda)$

→ $c(x, y, z, \lambda) = E(x, y, z, \lambda)*r(x, y, z, \lambda)$

Camera( c(x, y, z, λ) )

# Light is part of the EM wave

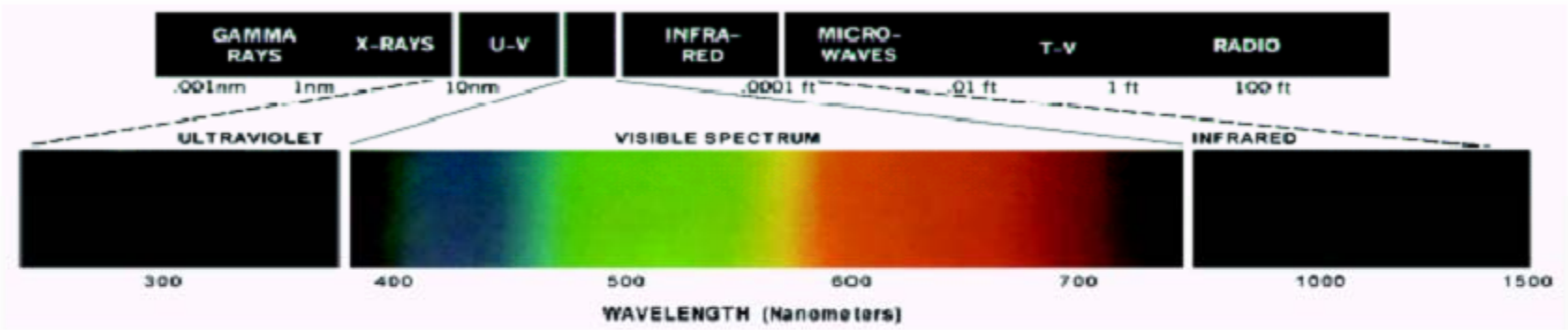
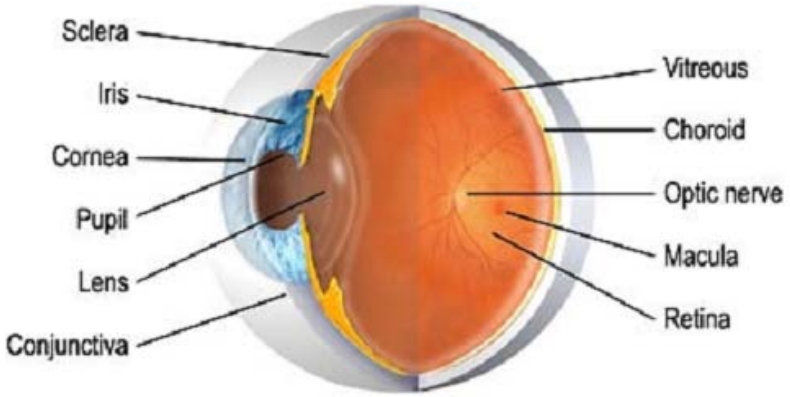



**FIGURE 6.2** Wavelengths comprising the visible range of the electromagnetic spectrum. (Courtesy of the General Electric Co., Lamp Business Division.)

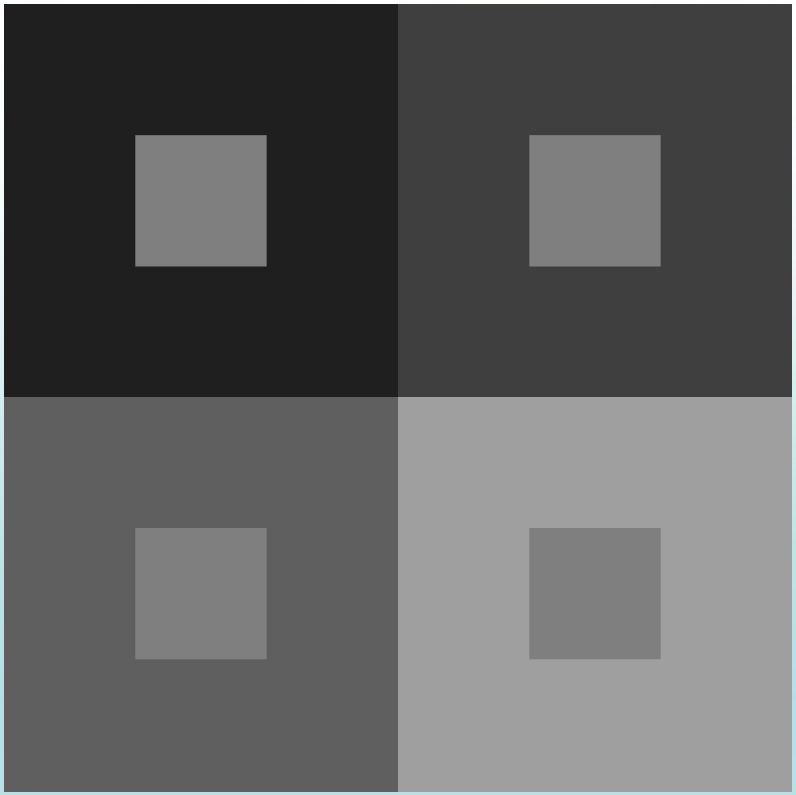
# What is an image?
## From physiology to mathematics

## Eye vs. Camera




| Camera components | Eye components |
|---|---|
| Lens | Lens, cornea |
| Shutter | Iris, pupil |
| Film | Retina |
| Cable to transfer images | Optic nerve send the info to the brain |

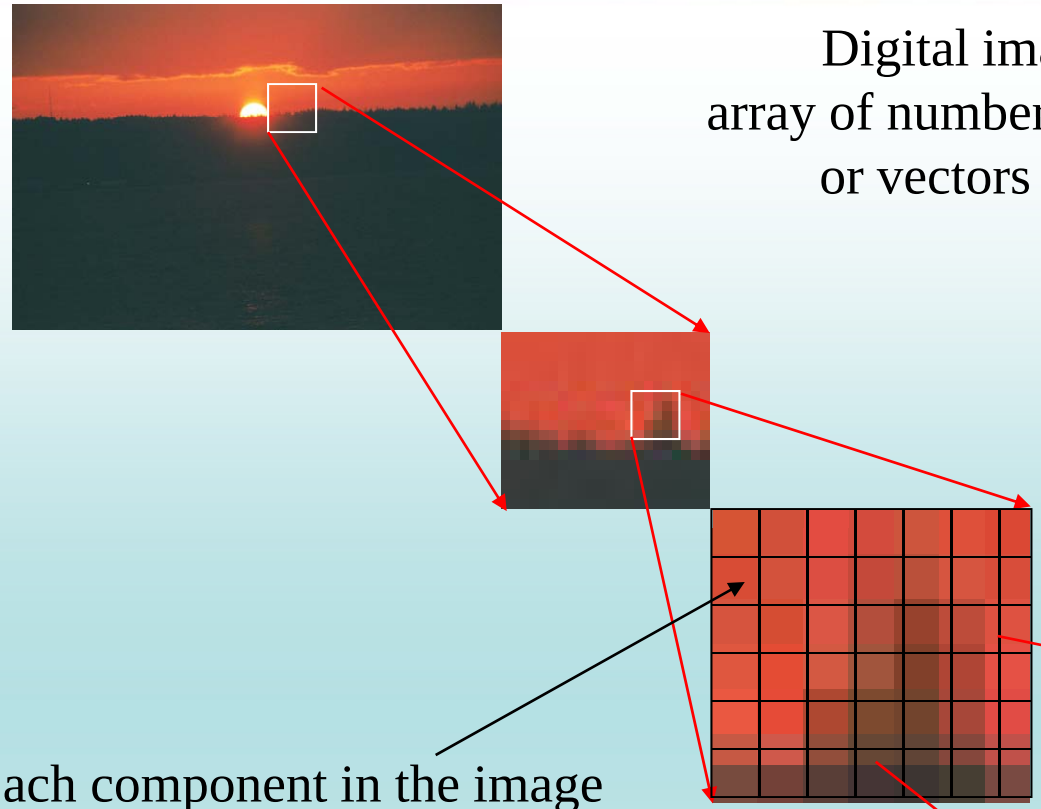
# What is an image?
## From physiology to mathematics



***Simultaneous contrast***. All small squares have exactly the same intensity but they appear progressively darker as background becomes lighter.

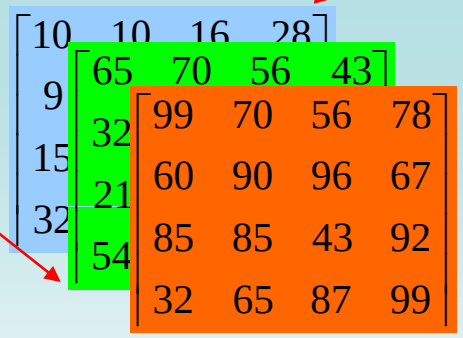
# What is an image?
## From physiology to mathematics

*Digital Image*



Digital image = a multidimensional array of numbers (such as intensity image) or vectors (such as color image)

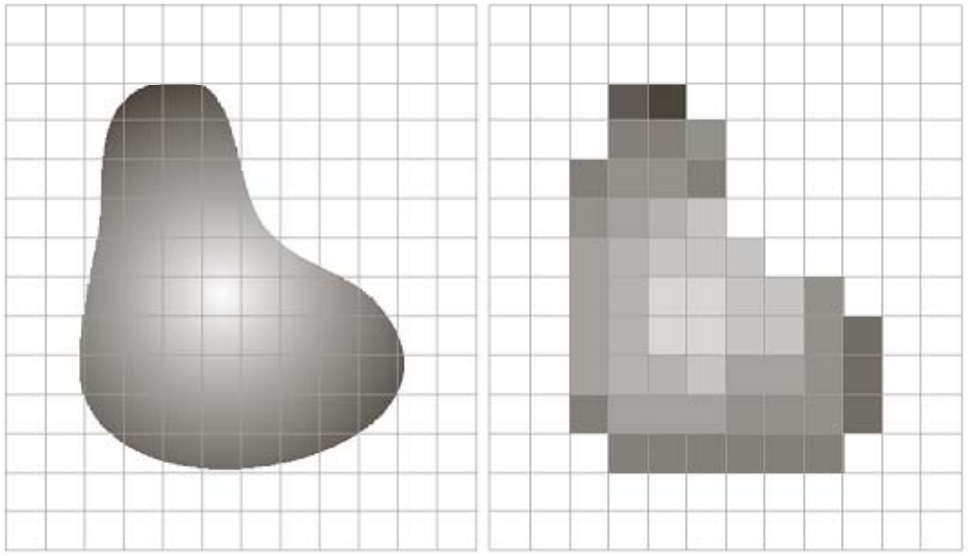
Each component in the image called pixel associates with the pixel value (a single number in the case of intensity images or a vector in the case of color images).



# What is an image?
From physiology to mathematics

## Analog to Digital Image Conversion

- Sampling: Dividing a continuous region into small squares (pixels), taking average value of each square
- Quantization: Map each value into one in a set of discrete values



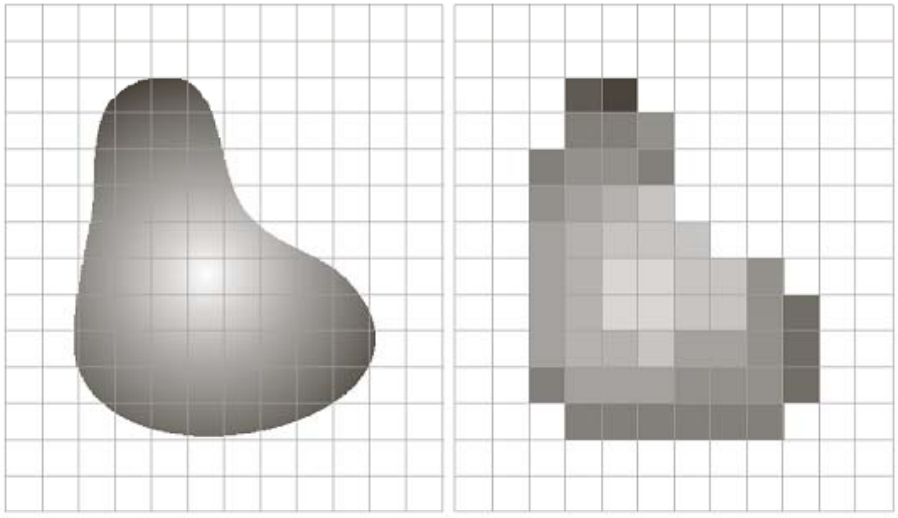
a b

**FIGURE 2.17** (a) Continuous image projected onto a sensor array. (b) Result of image sampling and quantization.

# What is an image?
## From physiology to mathematics

## Digital Image Capture by CCD Array

- Continuous Scene -> Digital image
  - Each CCD sensor averages the light intensity in a small region and output a discretized value



a b

**FIGURE 2.17** (a) Continuous image projected onto a sensor array. (b) Result of image sampling and quantization.

# **What is an image?**
From physiology to mathematics

## Grayscale Image Specification

- Each pixel value represents the brightness of the pixel. With 8-bit image, the pixel value of each pixel is 0 ~ 255
- Matrix representation: An image of MxN pixels is represented by an MxN array, each element being an unsigned integer of 8 bits

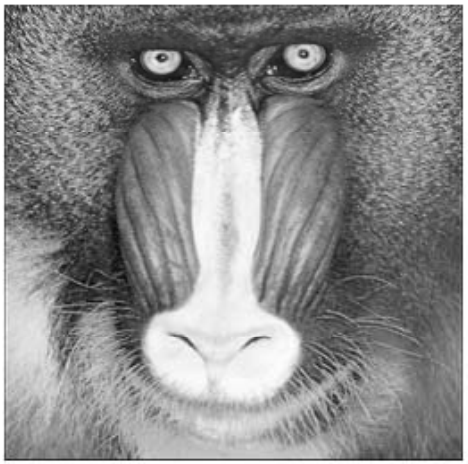
# What is an image?
## From physiology to mathematics

## Color Image Specification

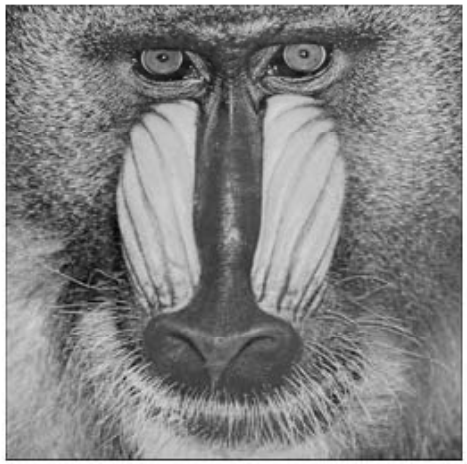
- Three components
  - M = {R, G, B}

$$R = \begin{bmatrix} 73 & \cdots & 87 \\ \vdots & \ddots & \vdots \\ 27 & \cdots & 17 \end{bmatrix}, G = \begin{bmatrix} 66 & \cdots & 98 \\ \vdots & \ddots & \vdots \\ 36 & \cdots & 13 \end{bmatrix}, B = \begin{bmatrix} 31 & \cdots & 61 \\ \vdots & \ddots & \vdots \\ 36 & \cdots & 14 \end{bmatrix}$$

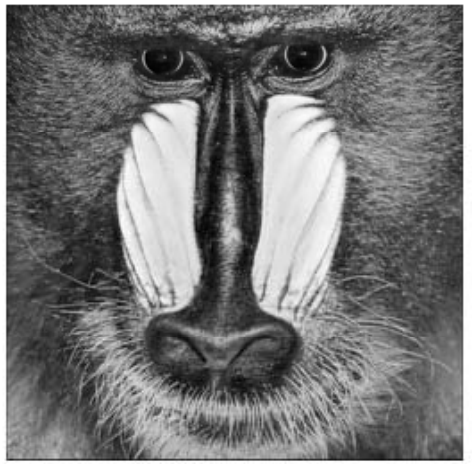
R



G



B

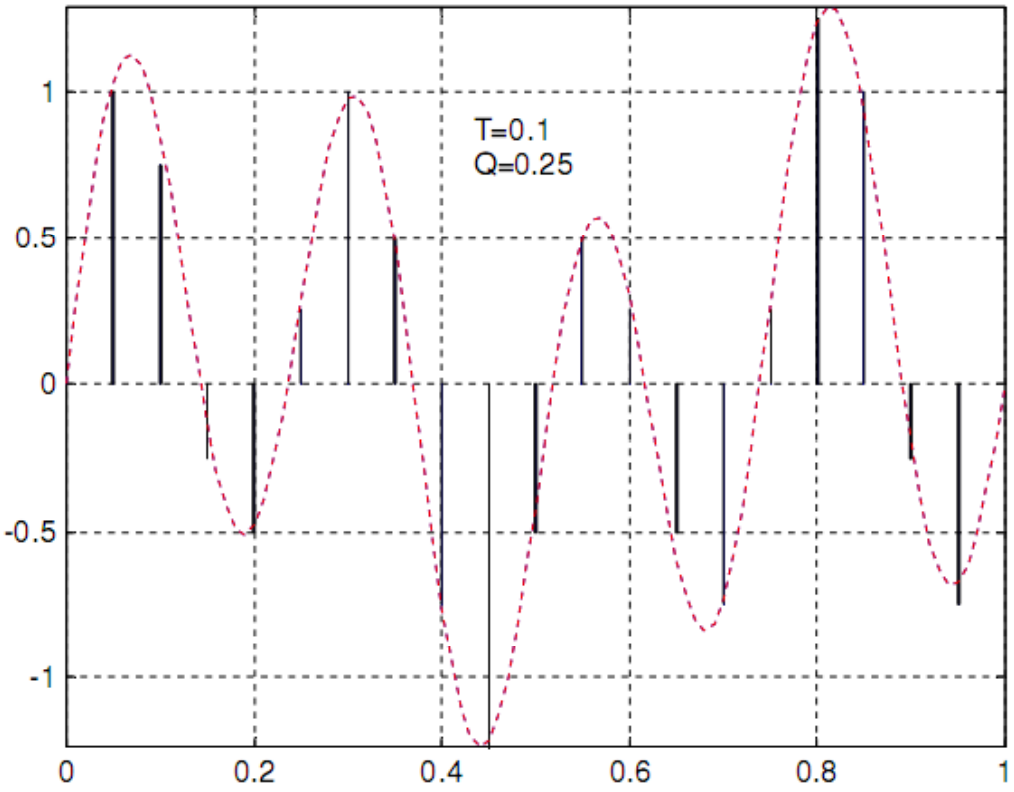


Red nose is brightest!

Blue Cheek is brightest

# What is an image?
From physiology to mathematics

## Analog to Digital Conversion



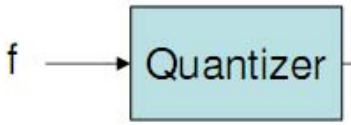

A2D_plot.m

# How to determine T and Q?

- $T$ (or $f_s$) depends on the signal frequency range
  - A fast varying signal should be sampled more frequently!
  - Theoretically governed by the Nyquist sampling theorem
    - $f_s > 2\, f_m$ ($f_m$ is the maximum signal frequency)
    - For speech: $f_s >= 8$ KHz; For music: $f_s >= 44$ KHz;
- $Q$ depends on the dynamic range of the signal amplitude and perceptual sensitivity
  - $Q$ and the signal range $D$ determine bits/sample $R$
    - $2^R = D/Q$
    - For speech and image: $R = 8$ bits; For music: $R = 16$ bits;
- One can trade off $T$ (or $f_s$) and $Q$ (or $R$)
  - lower $R$ -> higher $f_s$; higher $R$ -> lower $f_s$

# What is an image?
## From physiology to mathematics

## A Quantizer




Decision Levels $\{t_k, k = 0, \ldots, L\}$

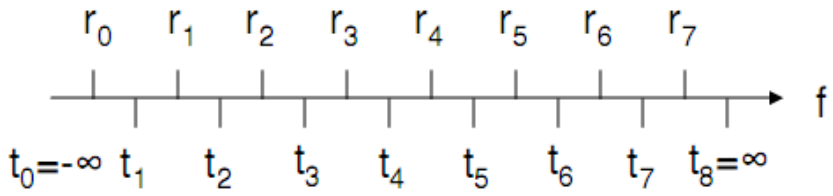
Reconstruction Levels $\{r_k, k = 0, \ldots, L-1\}$

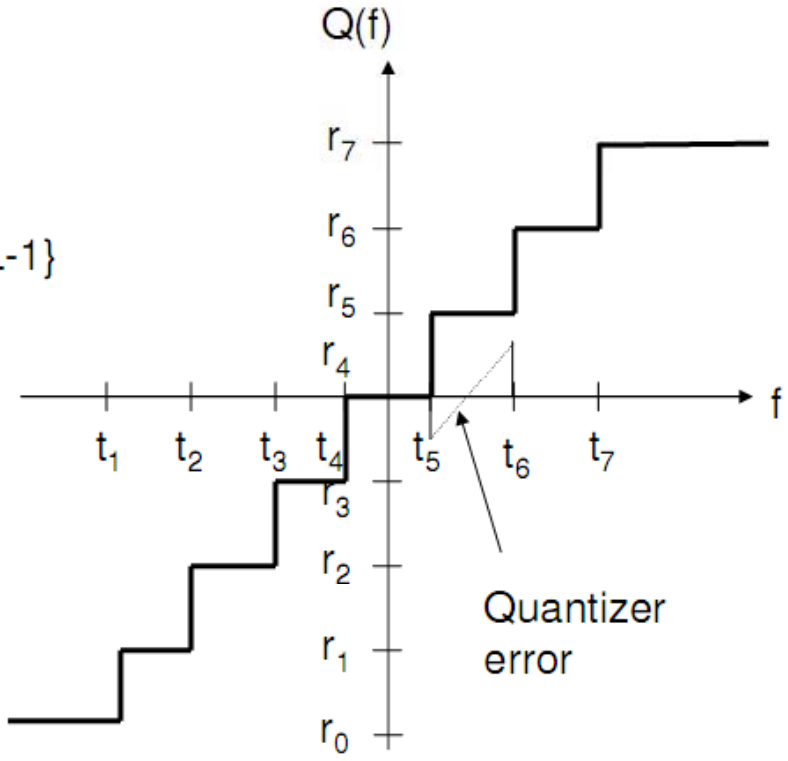
If $f \in [t_k, t_{k+1})$

Then $Q(f) = r_k$

L levels need $R = \lceil \log_2 L \rceil$ bits

$\lceil x \rceil$ returns the smallest integer that is bigger than or equal to x

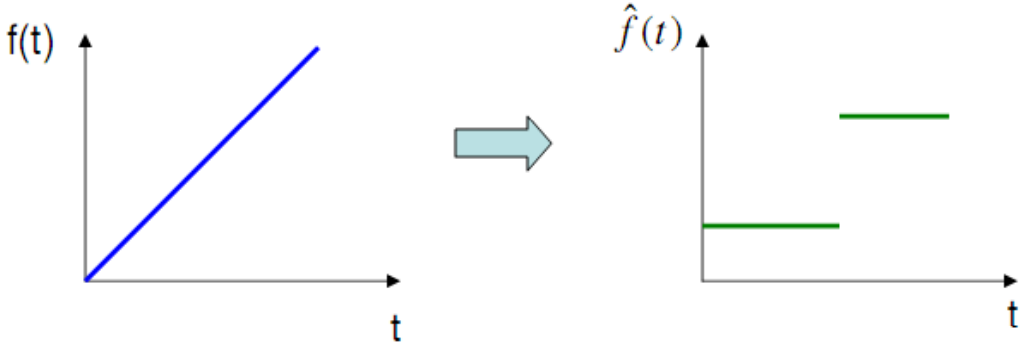


# What is an image?
From physiology to mathematics

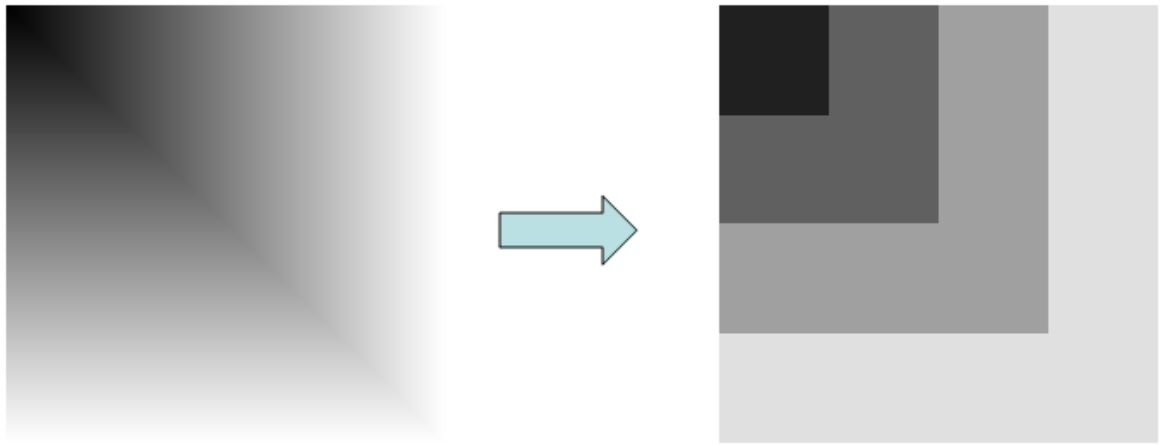
## Quantization Effect – False Contour

- 1-D Signal




- 2-D Image

```
x=zeros(256,256);
for I =1:256,
  for j = 1:256,
    x(i,j)=max(i,j)-1;
  end
end
Figure;
imshow(uint(x));
```



f(i,j)=max(I,j)

**What is an image?**
From physiology to mathematics

## Perception Optimized Quantization

- Reduce the artifact of false contour
- Better visual perception (Human eyes are very sensitive to edges)
- MSE might be worse

# What is an image?
From physiology to mathematics

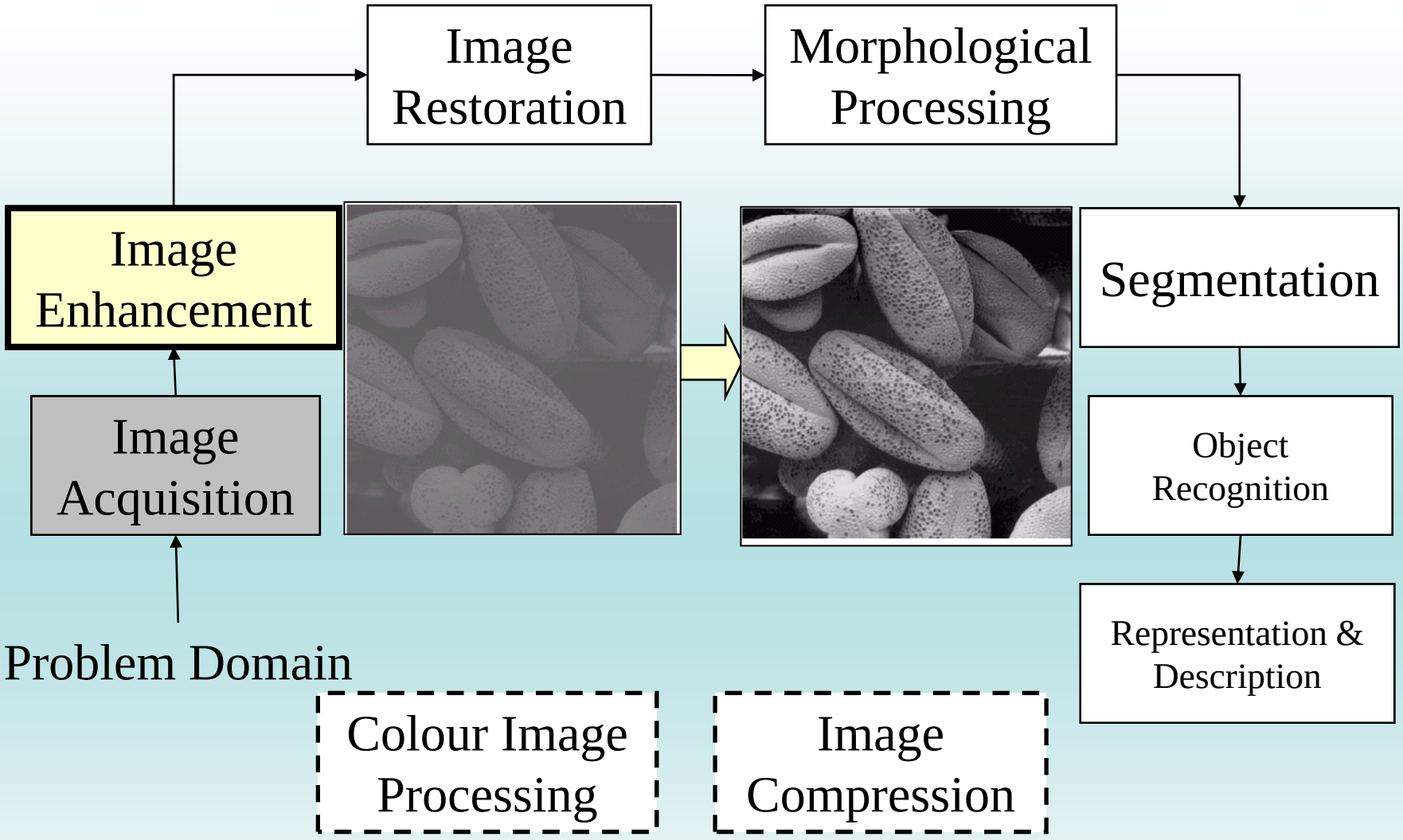
## Example of Color Quantization

24 bits -> 8 bits

Adaptive (non-uniform) quantization
(vector quantization)

Uniform quantization
(3 bits for R,G, 2 bits for B)

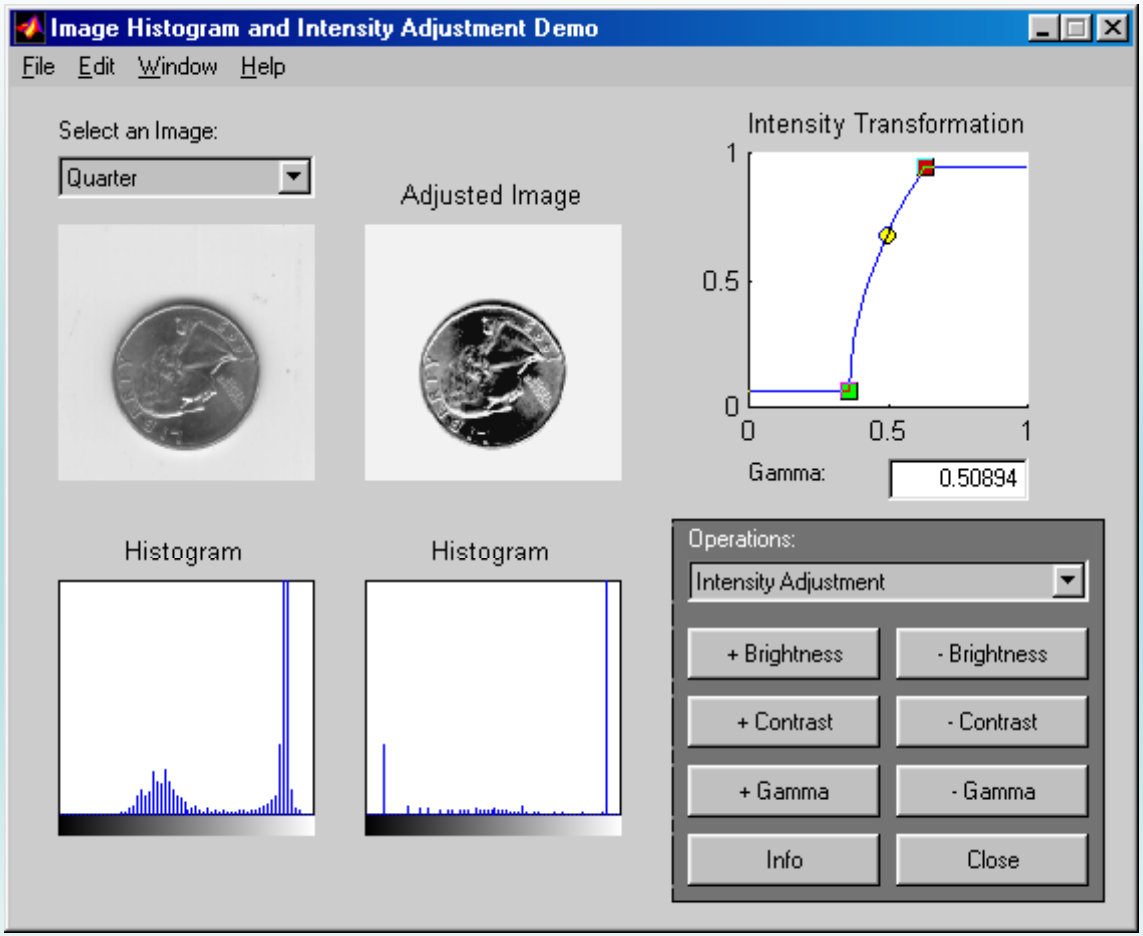
# Key Stages in Digital Image Processing: Image Enhancement



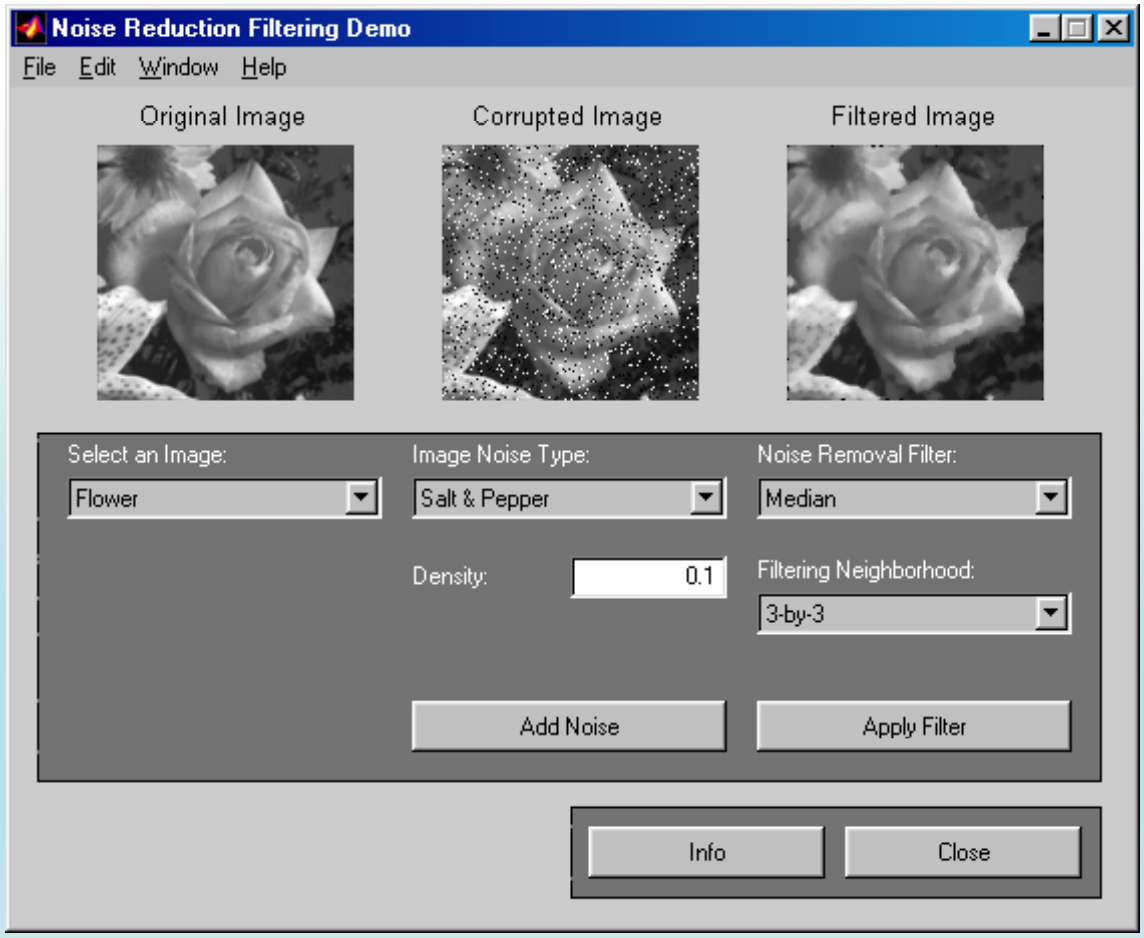
# Image Enhancement

- Enhancement in the spatial and frequency domains
  - Histograms
  - Denoising

# Contrast enhancement



# Noise reduction



# Noise Removal

- What is meant by noise removal?
- What is meant by a noise model?

$$g(x,y) = f(x,y) + \eta(x,y)$$

  - Common noise models
    - Gaussian
    - Rayleigh
    - Erlang
    - Exponential
    - Uniform
    - Impulse (salt & pepper)
- Filtering to remove noise
  - Simple mean filter
  - Other mean filters

# Spatial Filtering

- Spatial differentiation
  - 1st derivative $$\frac{\partial f}{\partial x} = f(x+1) - f(x)$$
  - 2nd derivative $$\frac{\partial^2 f}{\partial^2 x} = f(x+1) + f(x-1) - 2f(x)$$
- Differentiation based filters

| | | |
|---|---|---|
| 0 | 1 | 0 |
| 1 | -4 | 1 |
| 0 | 1 | 0 |

| | | |
|---|---|---|
| -1 | -2 | -1 |
| 0 | 0 | 0 |
| 1 | 2 | 1 |

| | | |
|---|---|---|
| -1 | 0 | 1 |
| -2 | 0 | 2 |
| -1 | 0 | 1 |

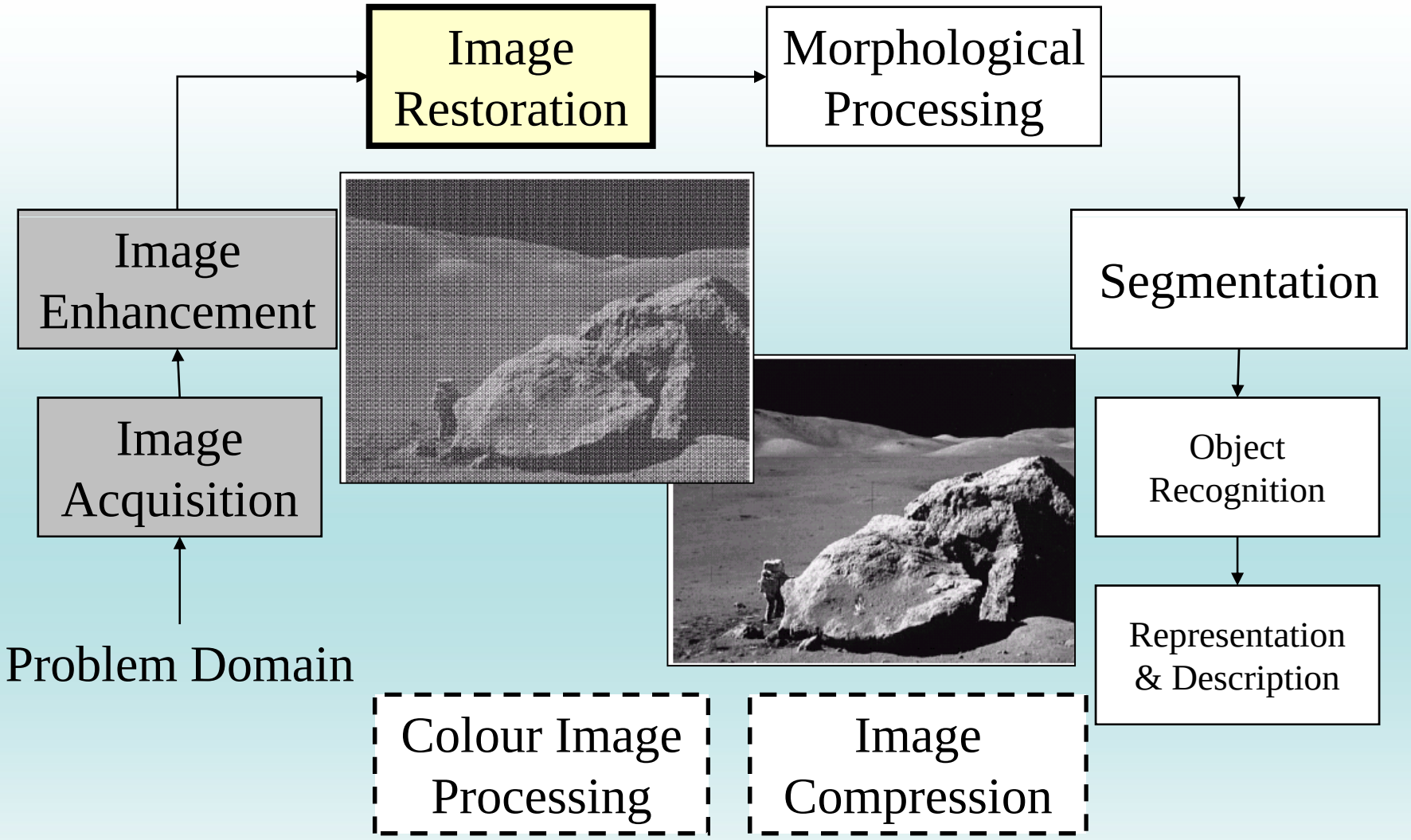
- How to do sharpening using these filters

Laplacian Sobel

# Frequency Domain Filtering

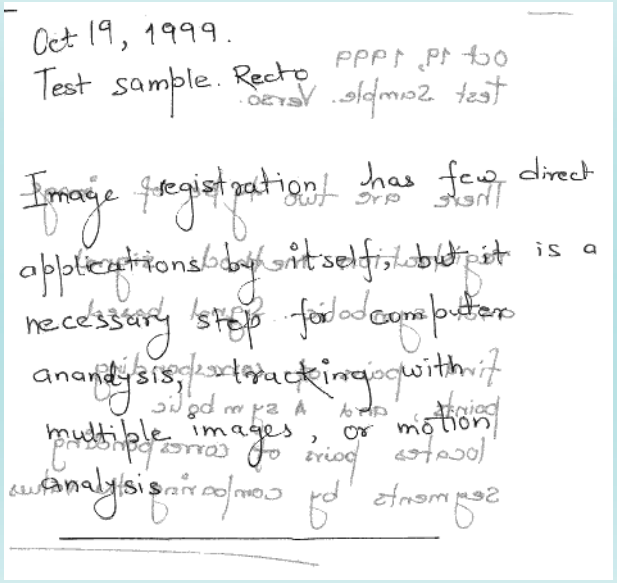
- The Fourier transform
- How filtering in the frequency domain works
- Low pass filters
  - What are they for?
  - Ideal low pass filter
  - Butterworth low pass filter
  - Gaussian low pass filter
- High pass filters
  - What are they for?
  - Ideal high pass filter
  - Butterworth high pass filter
  - Gaussian high pass filter

# Key Stages in Digital Image Processing: Image Restoration

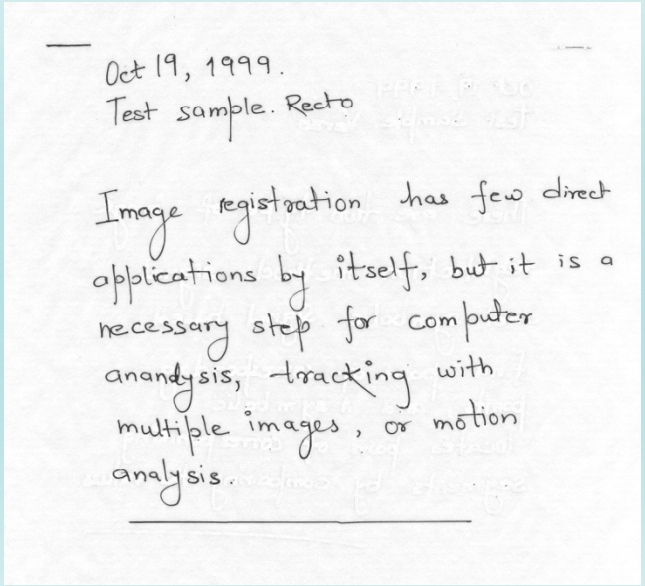


# Bleed-through Removal

- Replace detected bleed-through with estimate of background

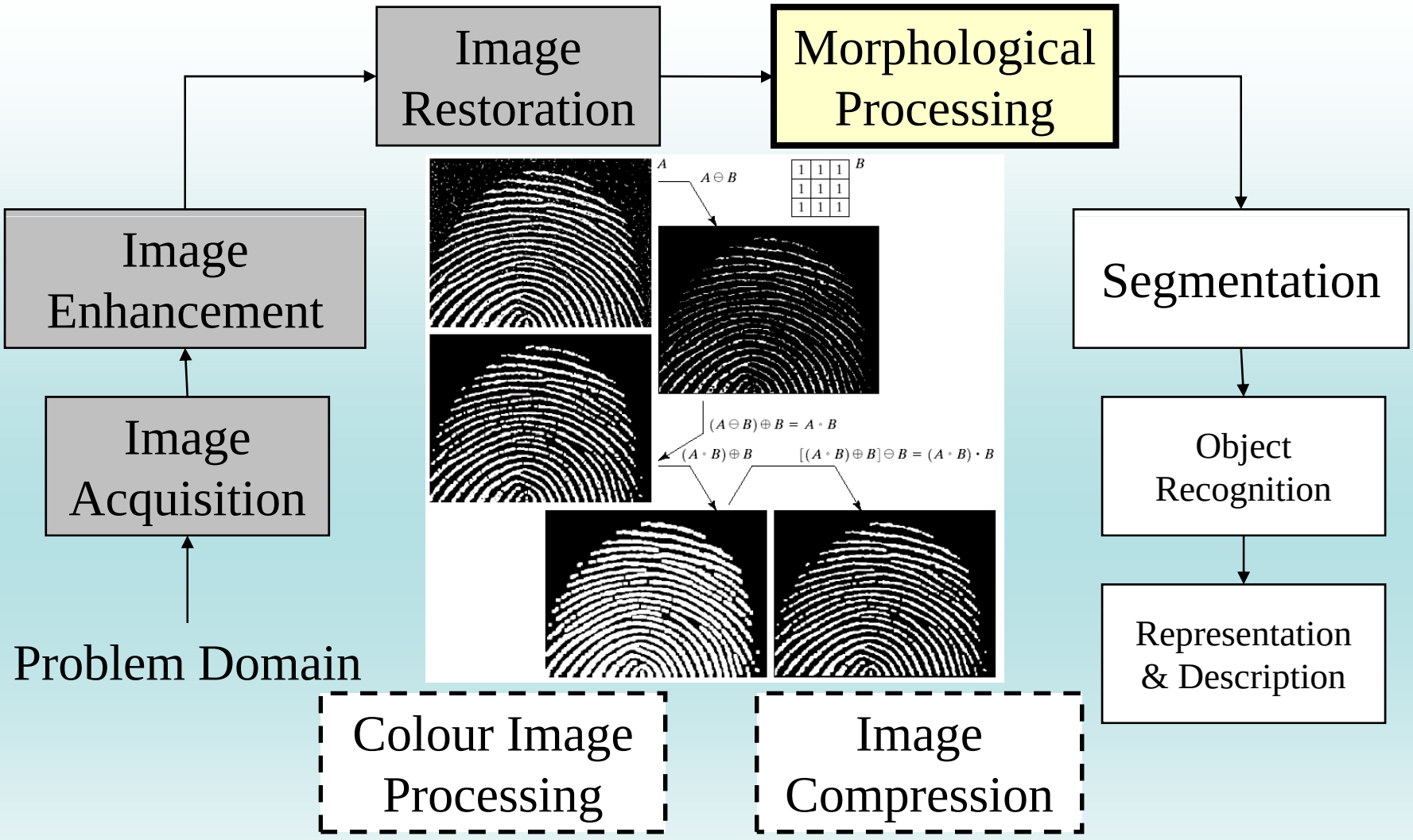



Before restoration

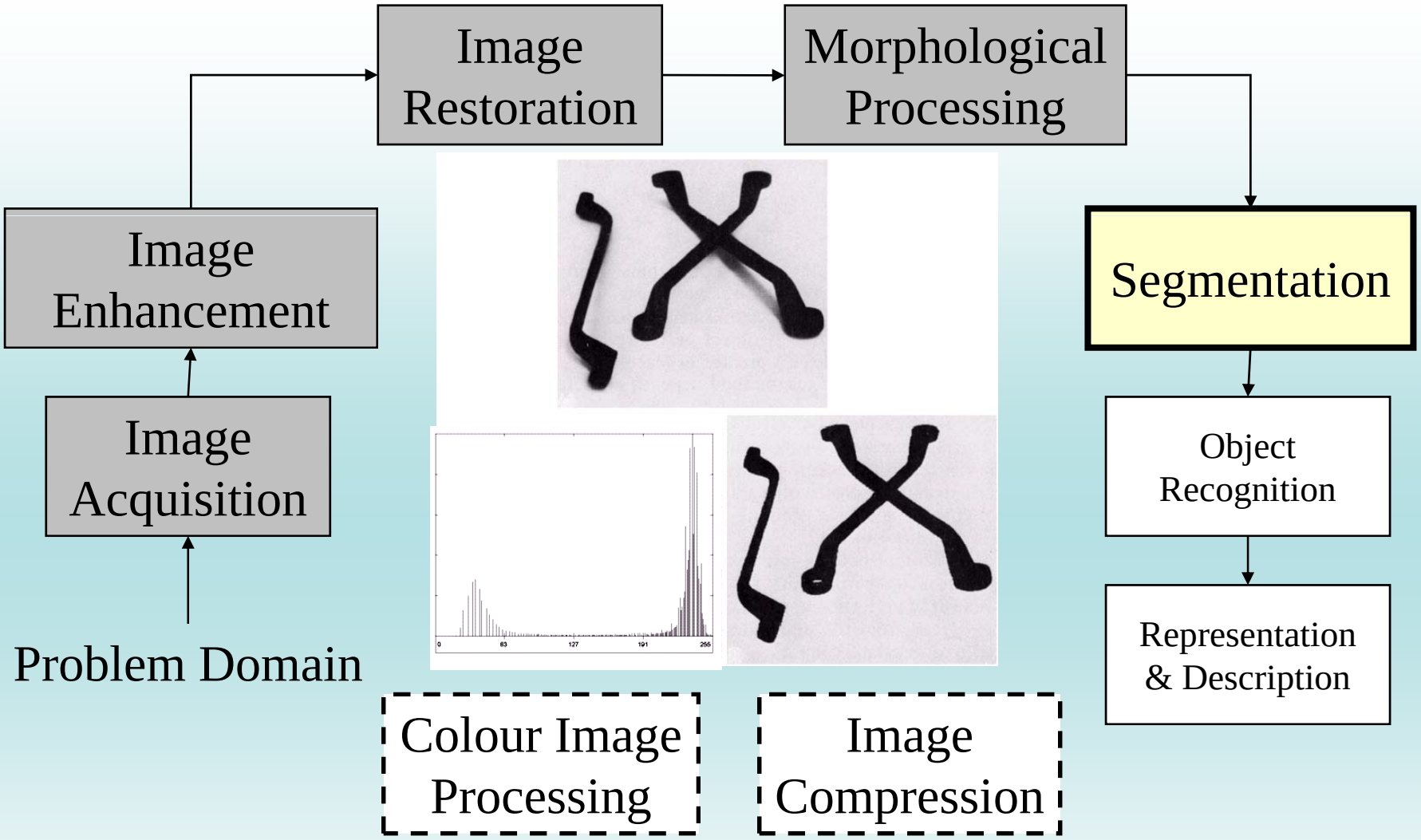



After restoration

# Key Stages in Digital Image Processing: Morphological Processing



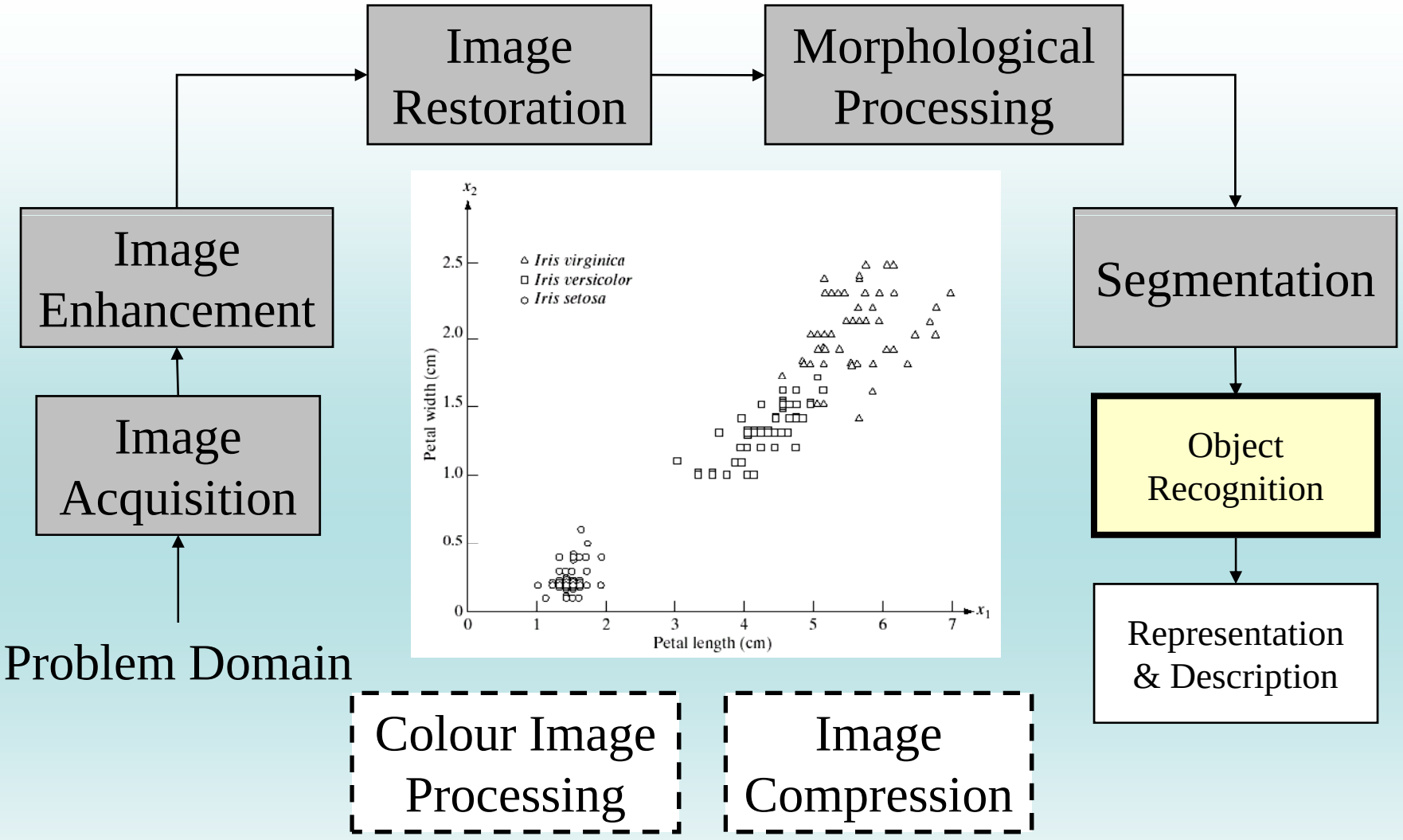
# Key Stages in Digital Image Processing: Segmentation



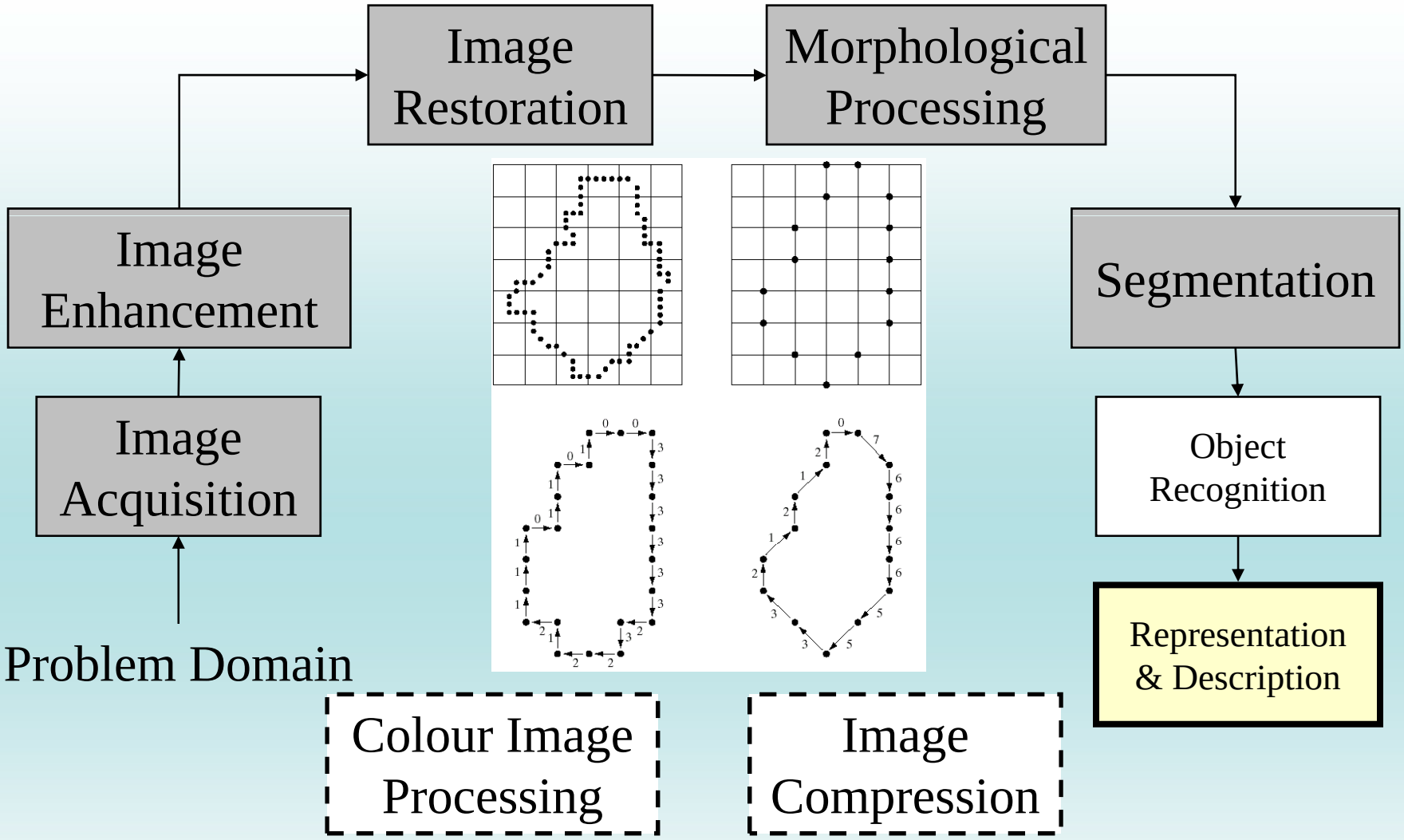
# Image Segmentation

- Segmentation of different object in the scene

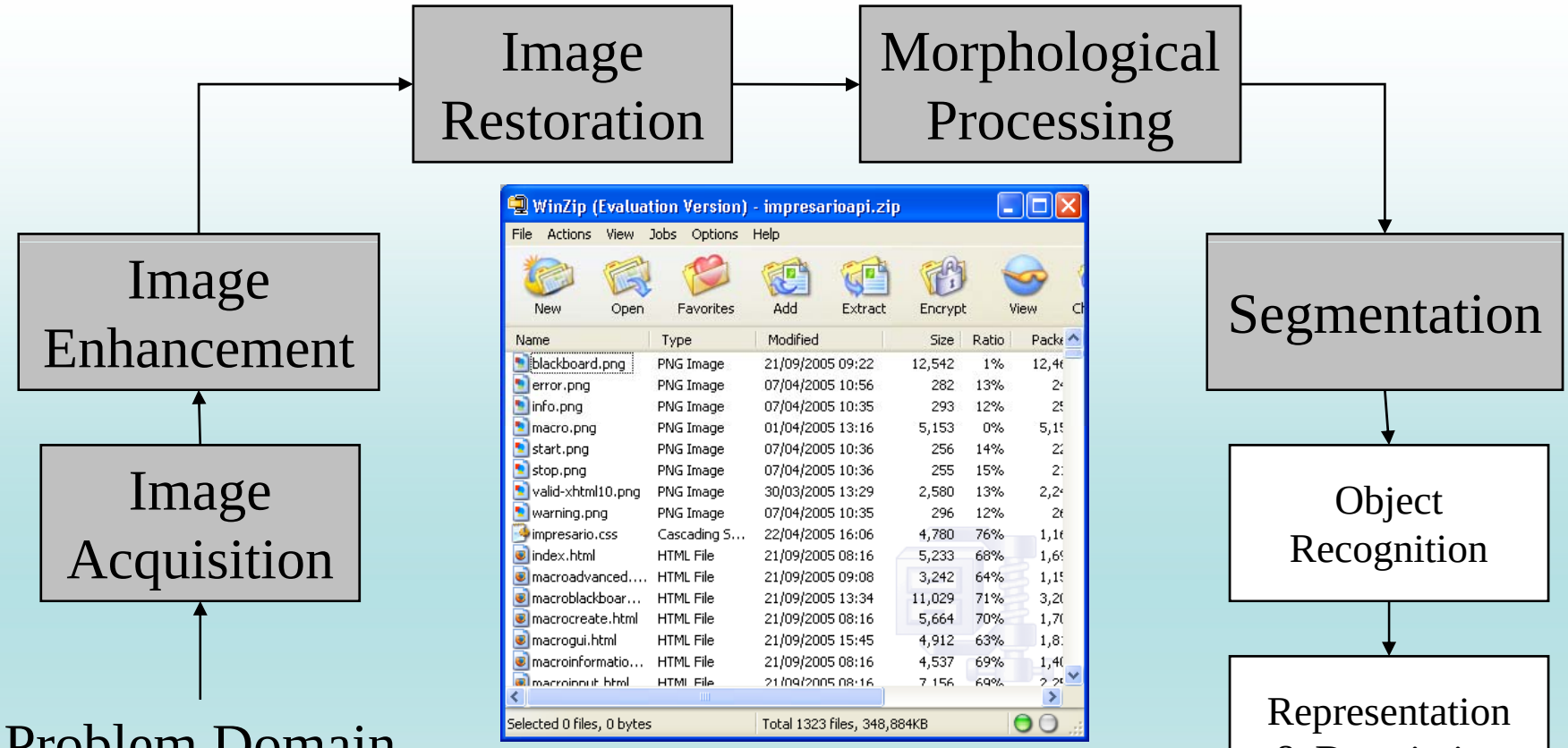
# Key Stages in Digital Image Processing: Object Recognition



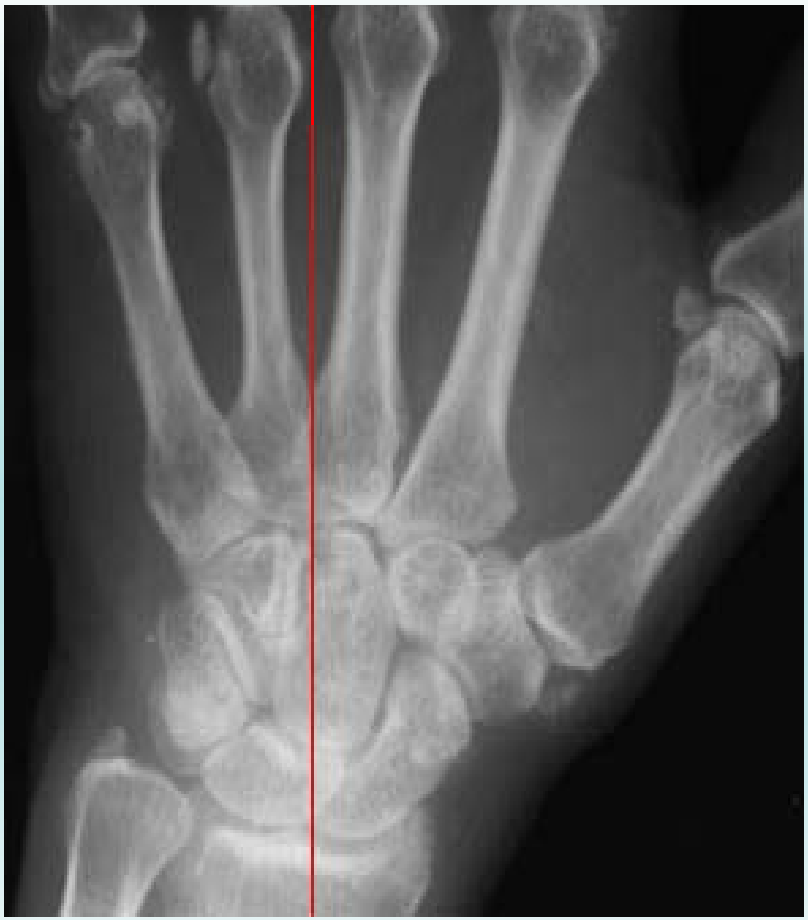
# Key Stages in Digital Image Processing: Representation & Description



# Key Stages in Digital Image Processing: Image Compression

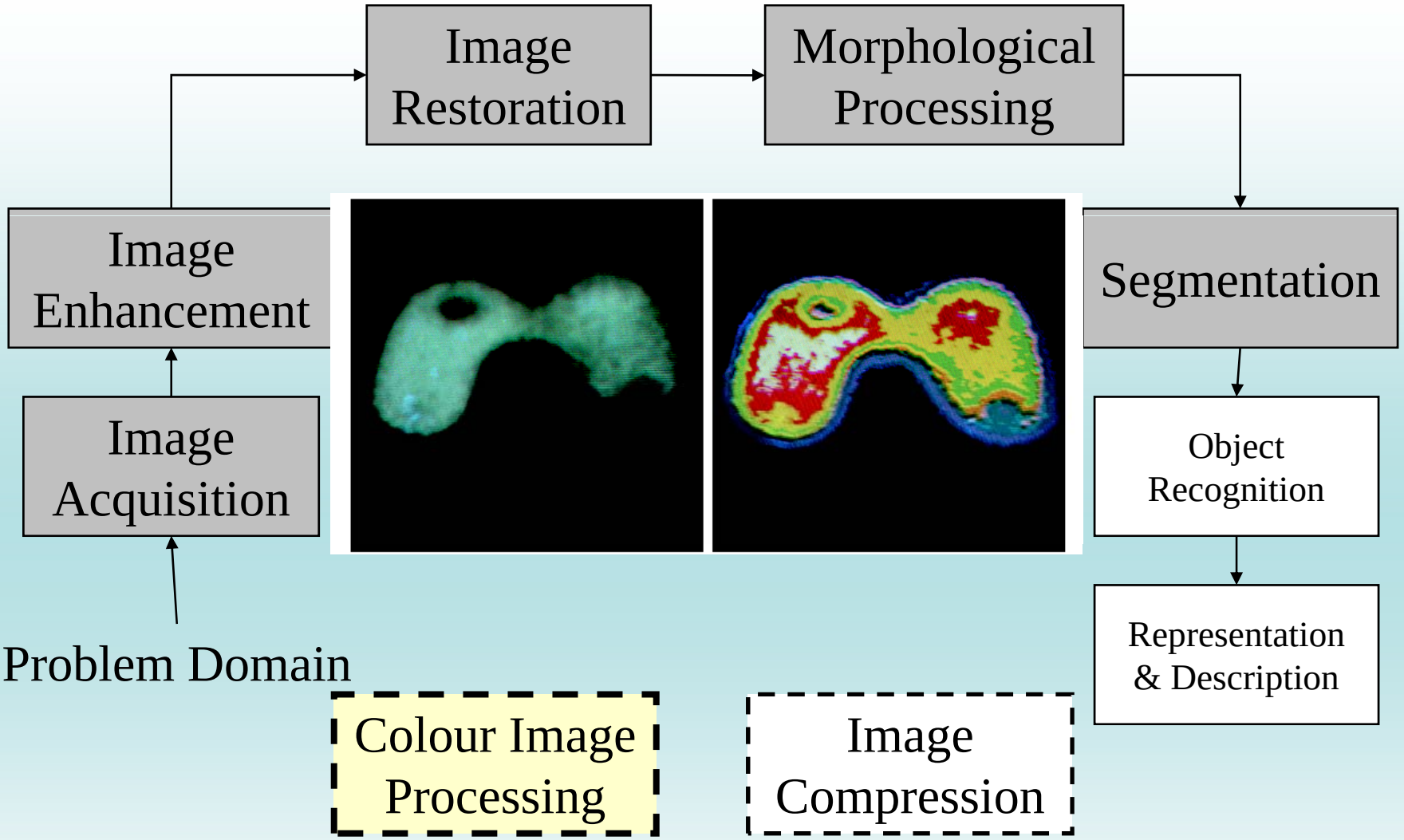


## Compression



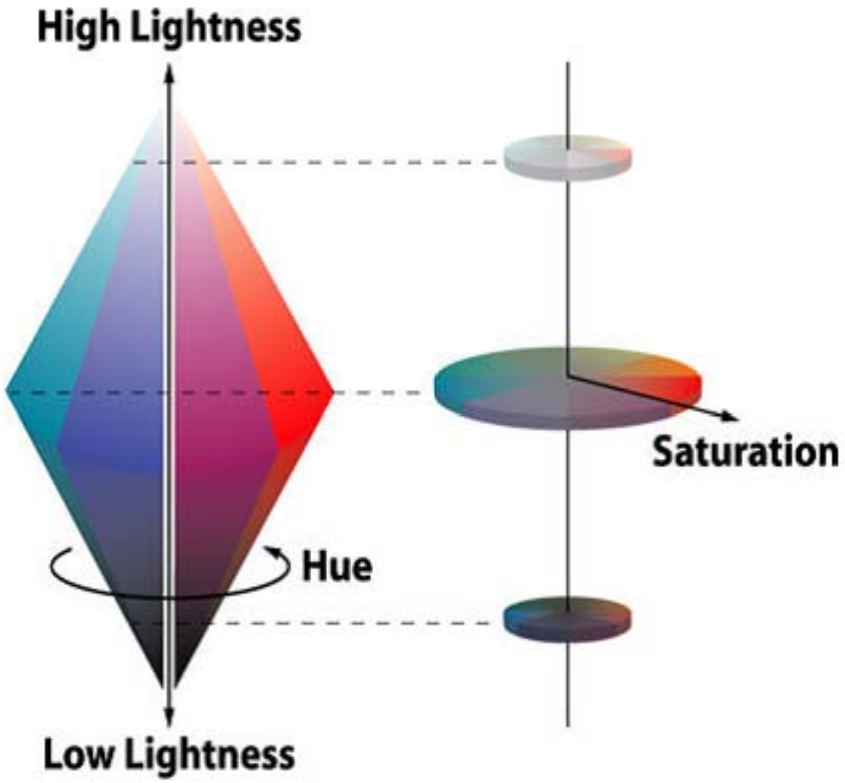
- Original: left side
- Compression Ratio of 100:1 no degradation is visible

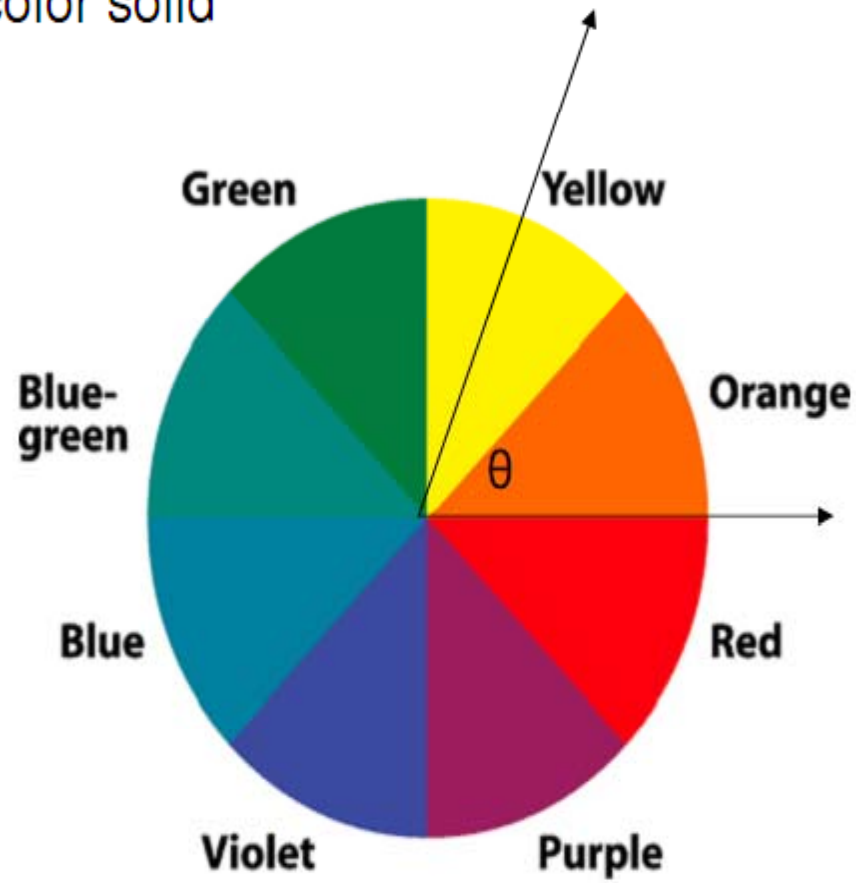
# Key Stages in Digital Image Processing: Colour Image Processing



# Three Attributes of Color

- Luminance (brightness)
- Chrominance
  - Hue (color tone) and Saturation (color purity)
- Represented by a “color cone” or “color solid”



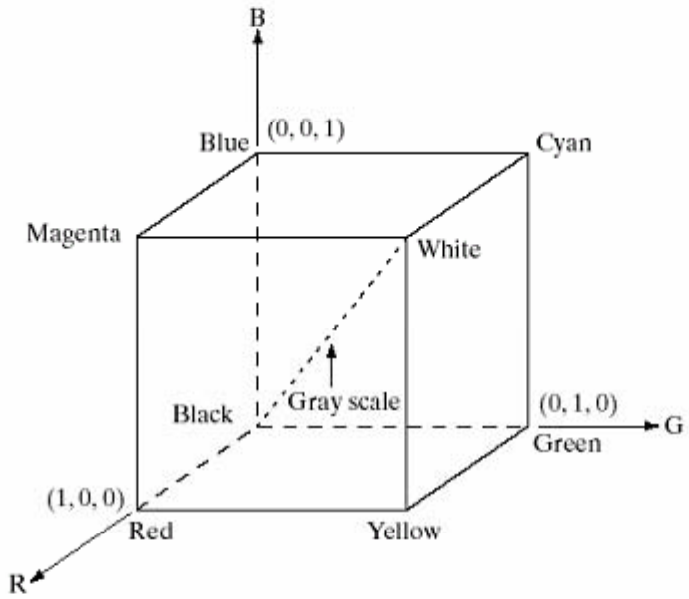



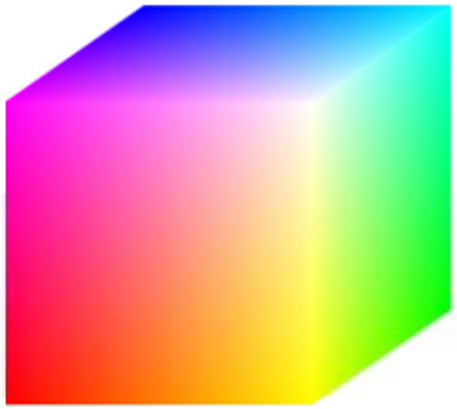
# HSI Color Model

- Hue represents dominant color as perceived by an observer. It is an attribute associated with the dominant wavelength.
- Saturation refers to the relative purity or the amount of white light mixed with a hue. The pure spectrum colors are fully saturated. Pink and lavender are less saturated.
- Intensity reflects the brightness.

# RGB Color Model

**FIGURE 6.7**
Schematic of the RGB color cube. Points along the main diagonal have gray values, from black at the origin to white at point (1, 1, 1).



RGB 24-bit color cube

# Tri-chromatic Color Mixing

- Tri-chromatic color mixing theory
  - Any color can be obtained by mixing three primary colors with a right proportion

# Color Representation

- Specify three primary colors directly
  - Red, Green, Blue (RGB)
  - Cyan, Magenta, Yellow (CMY)
- Specify the luminance and chrominance
  - HSB or HSI (Hue, saturation, and brightness or intensity)
  - YIQ (used in NTSC color TV)
  - YCbCr (used in digital color TV)
  - Can be determined from RGB or CMY
- Amplitude specification:
  - 8 bits per color component, or 24 bits per pixel
  - Total of 16 million colors
  - A 1kx1k true RGB color requires 3 MB memory

# CMY and CMYK Color Models

- Conversion between RGB and CMY (assuming maximum value is 1)

$$\begin{bmatrix} C \\ M \\ Y \end{bmatrix} = \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix} - \begin{bmatrix} R \\ G \\ B \end{bmatrix}, \quad \begin{bmatrix} R \\ G \\ B \end{bmatrix} = \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix} - \begin{bmatrix} C \\ M \\ Y \end{bmatrix}.$$

- Equal amounts of Cyan, Magenta, and Yellow produce black. In practice, this produce muddy-looking black. To produce true black, a fourth color, black is added, which is CMYK color model.

# Conversion Between RGB and HSI

- Converting color from RGB to HSI

$$H = \begin{cases} \theta & \text{if } B \le G \\ 360 - \theta & \text{if } B > G \end{cases} \quad \text{with } \theta = \cos^{-1}\left\{ \frac{\frac{1}{2}[(R-G)+(R-B)]}{[(R-G)^2+(R-B)(G-B)]^{\frac{1}{2}}} \right\}$$

$$S = 1 - \frac{3}{(R+G+B)}[\min(R,G,B)]$$

$$I = \frac{1}{3}[R+G+B]$$

- Converting color from HSI to RGB

RG sector ($0 \leqslant H < 120$)

$$B = I(1-S)$$

$$R = I\left[1 + \frac{S\cos H}{\cos(60-H)}\right]$$

$$G = 1-(R+B)$$

GB sector ($120 \leqslant H < 240$)

$$R = I(1-S)$$

$$G = I\left[1 + \frac{S\cos(H-120)}{\cos(60-(H-120))}\right]$$

$$B = 1-(R+G)$$

BR sector ($240 \leqslant H < 360$)

$$G = I(1-S)$$

$$B = I\left[1 + \frac{S\cos(H-240)}{\cos(60-(H-240))}\right]$$

$$R = 1-(G+B)$$

# YIQ Color Coordinate System

- YIQ is defined by the National Television System Committee (NTSC)
  - Y describes the luminance, I and Q describes the chrominance.
  - A more compact representation of the color.
  - YUV plays similar role in PAL and SECAM.
- Conversion between RGB and YIQ

$$\begin{bmatrix} Y \\ I \\ Q \end{bmatrix} = \begin{bmatrix} 0.299 & 0.587 & 0.114 \\ 0.596 & -0.274 & -0.322 \\ 0.211 & -0.523 & 0.311 \end{bmatrix} \begin{bmatrix} R \\ G \\ B \end{bmatrix}, \quad \begin{bmatrix} R \\ G \\ B \end{bmatrix} = \begin{bmatrix} 1.0 & 0.956 & 0.621 \\ 1.0 & -0.272 & -0.649 \\ 1.0 & -1.106 & 1.703 \end{bmatrix} \begin{bmatrix} Y \\ I \\ Q \end{bmatrix}$$

# YUV/YCbCr Coordinate

- YUV is the color coordinate used in color TV in PAL system, somewhat different from YIQ.
- YCbCr is the digital equivalent of YUV, used for digital TV, with 8 bit for each component, in range of 0-255

$$\begin{bmatrix} Y \\ C_b \\ C_r \end{bmatrix} = \begin{bmatrix} 0.257 & 0.504 & 0.098 \\ -0.148 & -0.291 & 0.439 \\ 0.439 & -0.368 & -0.071 \end{bmatrix} \begin{bmatrix} R \\ G \\ B \end{bmatrix} + \begin{bmatrix} 16 \\ 128 \\ 128 \end{bmatrix}$$

$$\begin{bmatrix} R \\ G \\ B \end{bmatrix} = \begin{bmatrix} 1.164 & 0.000 & 1.596 \\ 1.164 & -0.392 & -0.813 \\ 1.164 & 2.017 & 0.000 \end{bmatrix} \begin{bmatrix} Y - 16 \\ C_b - 128 \\ C_r - 128 \end{bmatrix}$$

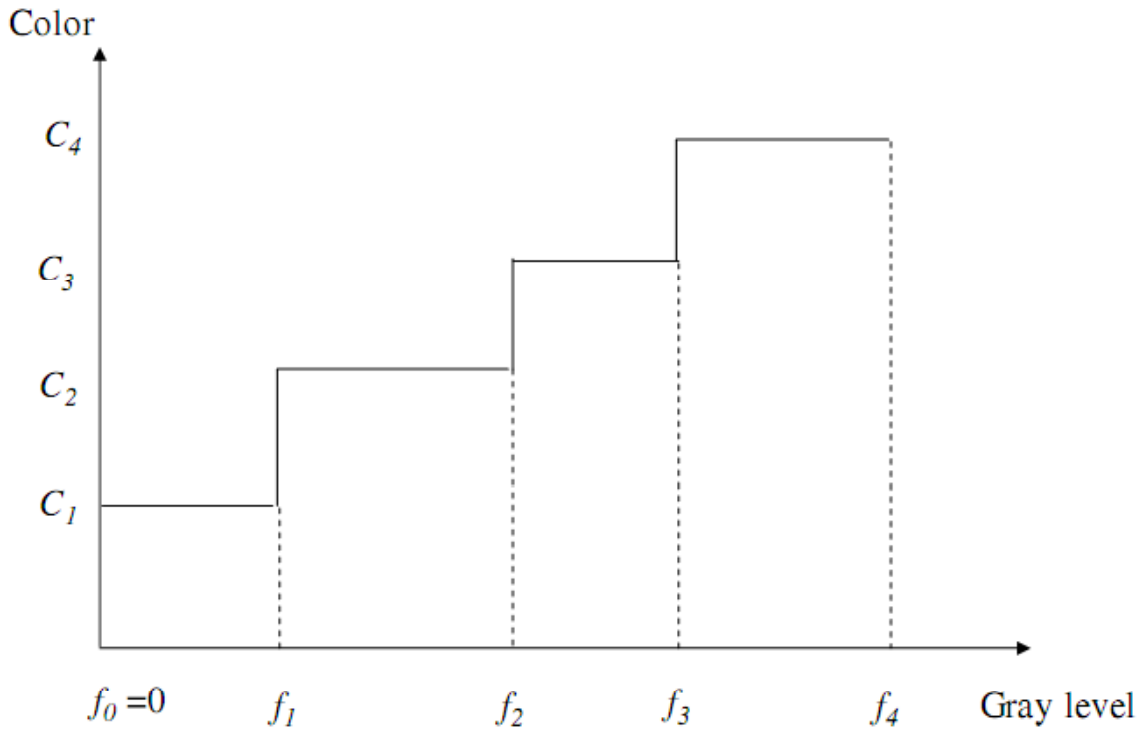
# Criteria for Choosing the Color Coordinates

- The type of representation depends on the applications at hand.
  - For display or printing, choose primary colors so that more colors can be produced. E.g. RGB for displaying and CMY for printing.
  - For analytical analysis of color differences, HSI is more suitable.
  - For transmission or storage, choose a less redundant representation, eg. YIQ or YUV or YCbCr

# Pseudo Color Display

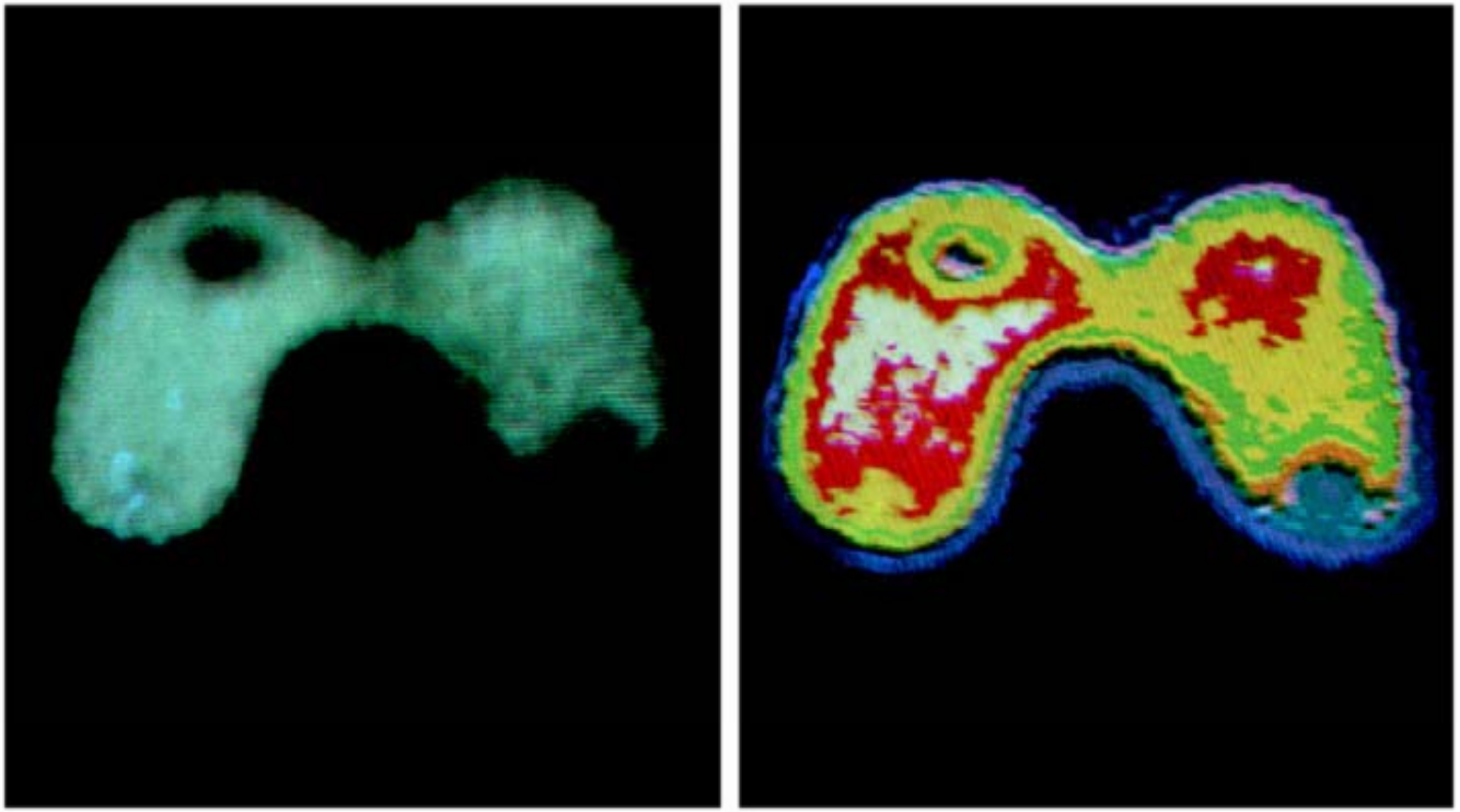
- **Intensity slicing:** Display different gray levels as different colors
  - Can be useful to visualize medical / scientific / vegetation imagery
    - E.g. if one is interested in features with a certain intensity range or several intensity ranges
- **Frequency slicing:** Decomposing an image into different frequency components and represent them using different colors.

# Intensity Slicing




Pixels with gray-scale (intensity) value in the range of $(f_{i-1}, f_i)$ are rendered with color $C_i$

# Example



a b

**FIGURE 6.20** (a) Monochrome image of the Picker Thyroid Phantom. (b) Result of density slicing into eight colors. (Courtesy of Dr. J. L. Blankenship, Instrumentation and Controls Division, Oak Ridge National Laboratory.)

# Pseudo Color Display of Multiple Images

- Display multi-sensor images as a single color image
  - Multi-sensor images: e.g. multi-spectral images by satellite

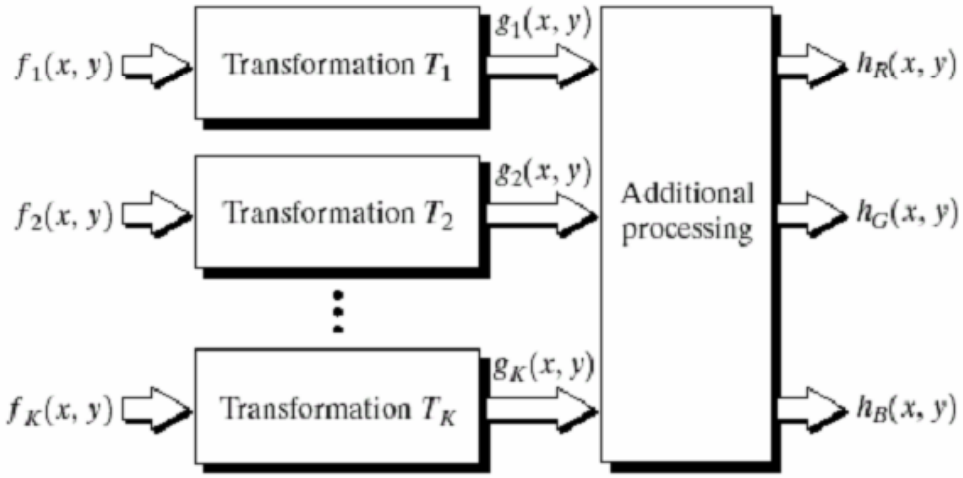
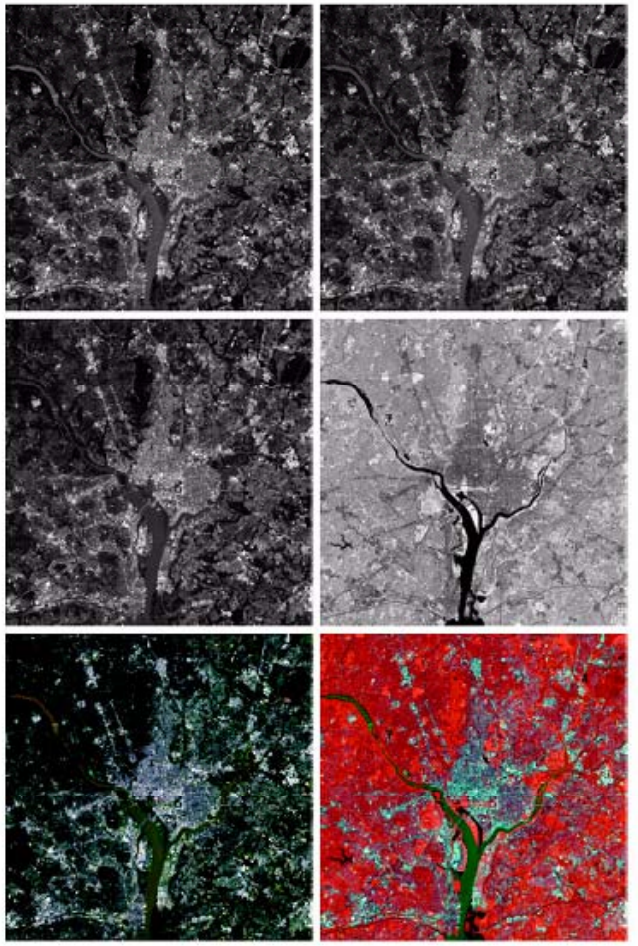



**FIGURE 6.26** A pseudocolor coding approach used when several monochrome images are available.

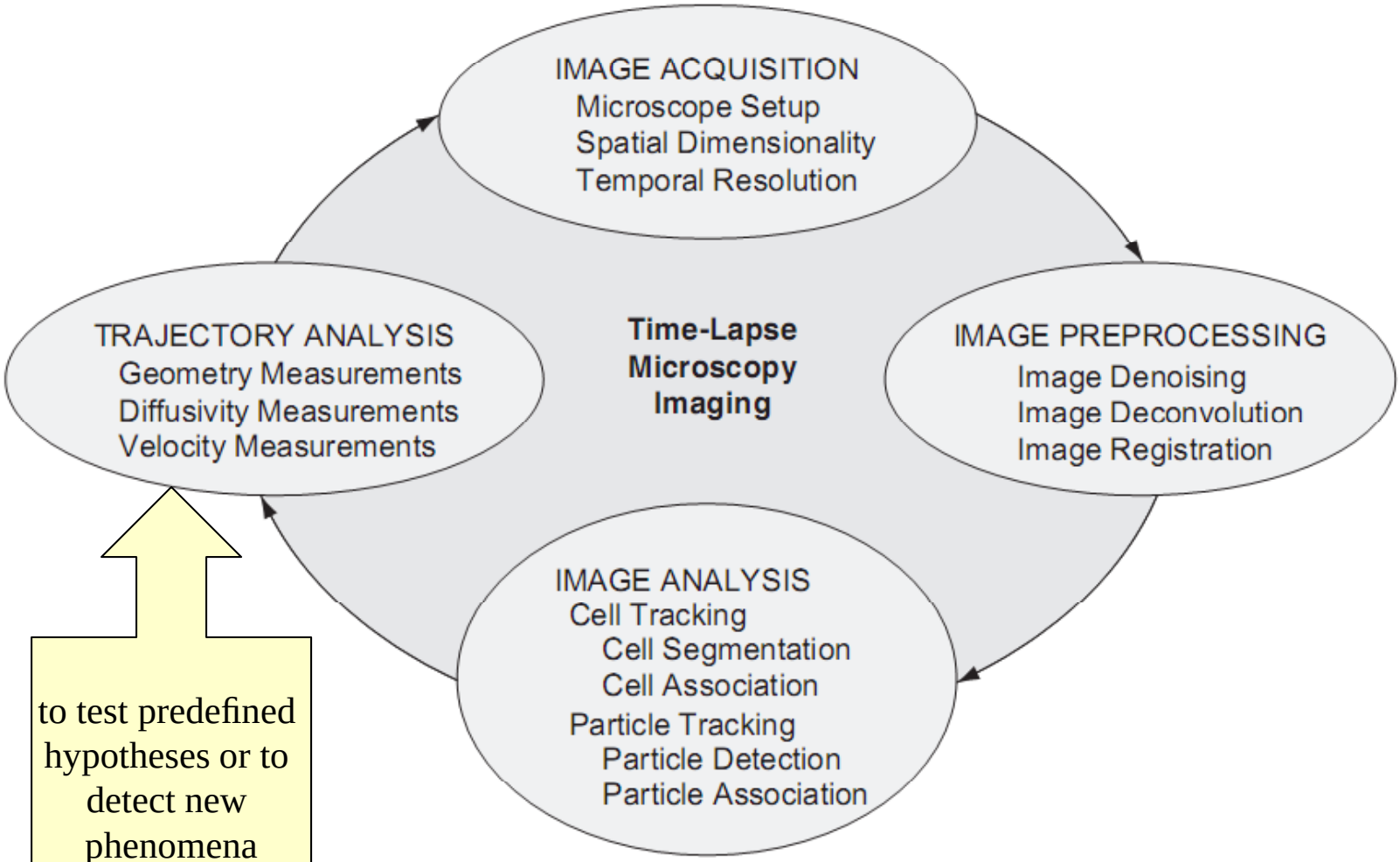
# An Example

a b
c d
e f

**FIGURE 6.27** (a)–(d) Images in bands 1–4 in Fig. 1.10 (see Table 1.1). (e) Color composite image obtained by treating (a), (b), and (c) as the red, green, blue components of an RGB image. (f) Image obtained in the same manner, but using in the red channel the near-infrared image in (d). (Original multispectral images courtesy of NASA.)



# Cell tracking



# Image acquisition

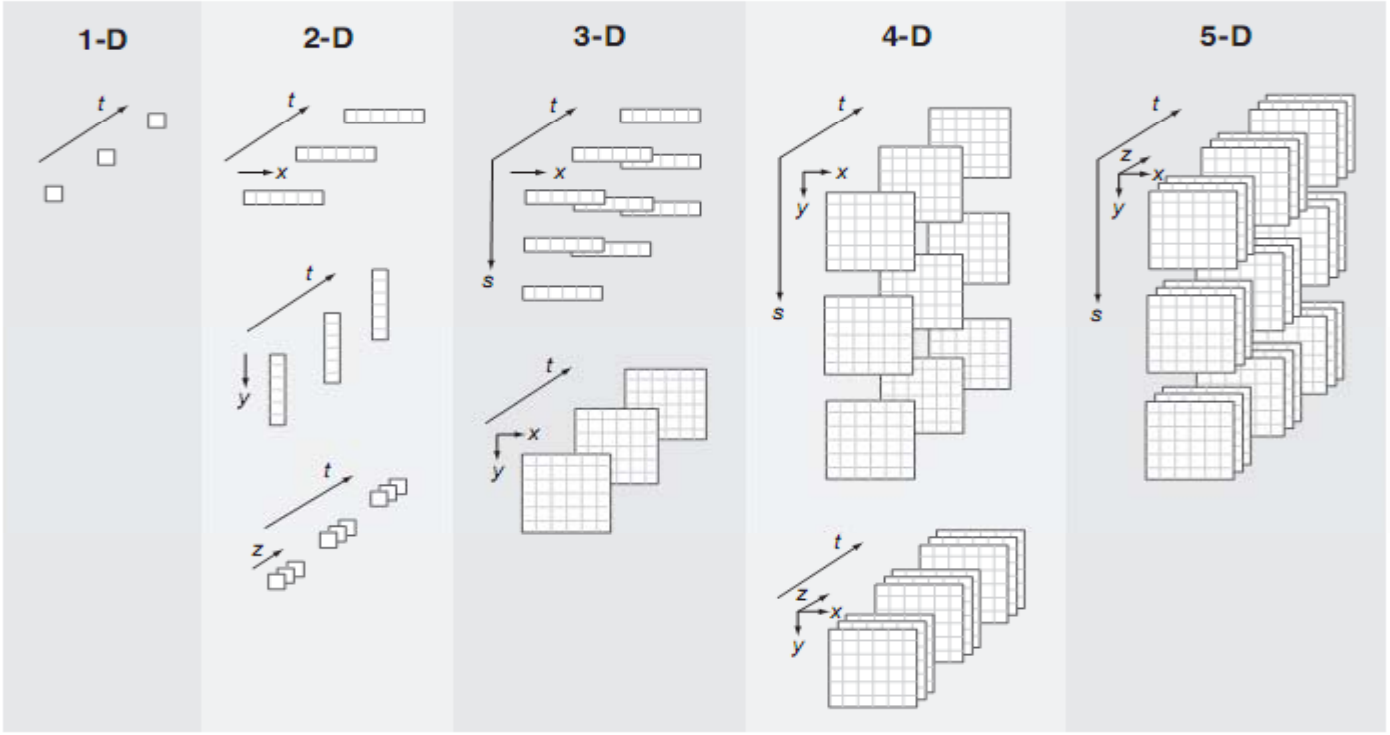



**FIGURE 15.3** Possible image configurations and dimensionalities in time-lapse microscopy imaging. Each dimension corresponds to an independent physical parameter or coordinate: *x* and *y* commonly denote the in-plane spatial coordinates, *z* the depth or axial coordinate, and *t* the time coordinate, and here *s* denotes any spectral parameter, such as wavelength. Notice that dimensionality, as used here, does not necessarily describe the image configuration unambiguously. For example, 4-D imaging may refer either to spatially 2-D multispectral time-lapse imaging or to spatially 3-D time-lapse imaging. To avoid confusion, it is better to use the abbreviations 2-D and 3-D to refer to spatial dimensionality only and to indicate explicitly whether the data also involves a temporal or spectral coordinate. Therefore, in this chapter we indicate spatially 2-D and 3-D time-lapse imaging by 2-D+t and 3-D+t respectively, rather than by 3-D and 4-D.

- Time-lapse imaging experiments generally involve living cells and organisms. A fundamental concern is keeping the specimen alive during the acquisition of hundreds or thousands of images over a period of time that may range from minutes to hours. This not only calls for a suitable environment with controlled temperature, humidity, and a stably buffered culture medium, but it also requires economizing light exposure, since living cells are subject to photo-damage. In fluorescence microscopy, excessive illumination bleaches fluorophores, and this limits their emission time span and generates free radicals that are toxic for living cells.

Type of the microscopy

- Two very important factors determine whether automated methods can be applied successfully, and they strongly affect accuracy. They are signal contrast (the intensity difference between objects and background) and noise, which, in light microscopy, is signal dependent. These two factors are usually combined into a single measure, the signal-to-noise ratio (SNR), calculated as the difference in mean intensity between the object, I0, and the background, Ib, divided by a representative noise level, s, that is, SNR=(I0-Ib)/s. Ideally, experiments should be designed so as to maximize SNR to allow robust and accurate automated image analysis, and the only way to accomplish this is with high light exposure levels.

# Image preprocessing

- Image Denoising
- Image Deconvolution
- Image Registration

One of the difficulties frequently encountered in quantitative motion analysis is the presence of unwanted movements confounding the movements of interest. In time-lapse imaging of living specimens, the observed movements are often a combination of global displacements and deformations of the specimen as a whole, superposed on the local movements of the structures of interest. For example, in intravital microscopy studies, which involve living animals, the image sequences may show cardiac, respiratory, or other types of global motion artifacts. But even in the case of imaging live-cell cultures, the dynamics of intracellular structures may be obscured by cell migration, deformation, or division. In these situations, prior motion correction is necessary. This can be achieved by global or local image alignment, also referred to as image registration.

# Image analysis (Cell tracking)

- Cell tracking methods generally consist of two main image processing steps:

  (1) cell segmentation (the spatial aspect of tracking), and

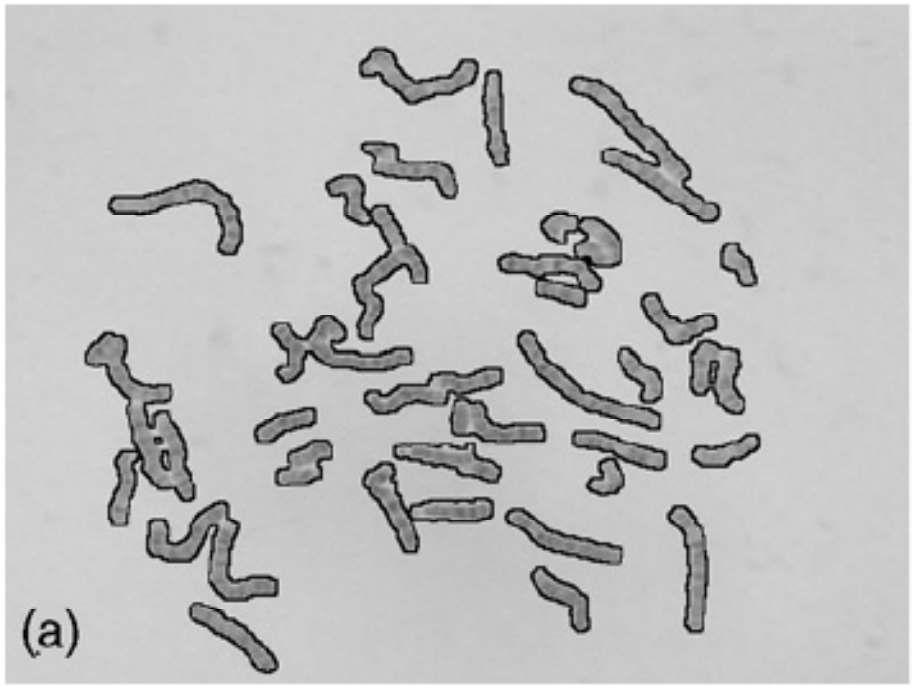
  (2) cell association (the temporal aspect).

- Segmentation is the process of dividing an image into (biologically) meaningful parts (segments), resulting in a new image containing for each pixel a label indicating to which segment it belongs (such as “foreground” versus “background”).

# Cell segmentation

- The simplest approach for separating cells from the background is intensity thresholding

$$G(x, y) = \begin{cases} F, & \text{if } I(x, y) \geq T \\ B, & \text{if } I(x, y) < T \end{cases}$$



It will be successful only if cells are well separated and their intensities differ sufficiently and consistently from the background—a condition hardly ever met in live-cell imaging due to severe noise, autofluorescence and photobleaching (in the case of fluorescence microscopy), or strongly varying intensities and halos (in the case of phase- or differential interference contrast microscopy).

- Fitting predetermined cell intensity profiles (templates) to the image data. This template matching approach works well for images showing consistent cell shape, but fails in the case of significant variations in cell morphology (between cells per image, or per cell over time, or both).

- Watersheds
- deformable models
- Starting from a coarse, initial segmentation, deformable models are iteratively evolved in the image domain to minimize a predefined energy functional. The modeling aspect lies primarily in the definition of this energy functional. Typically it consists of image-related terms (based on image features such as intensity, gradients, and texture) and image-independent terms (based on shape properties such as boundary length or surface area, curvature, and similarity to reference shapes). This mixture of terms enables the incorporation of both image information and prior knowledge about the biological application.

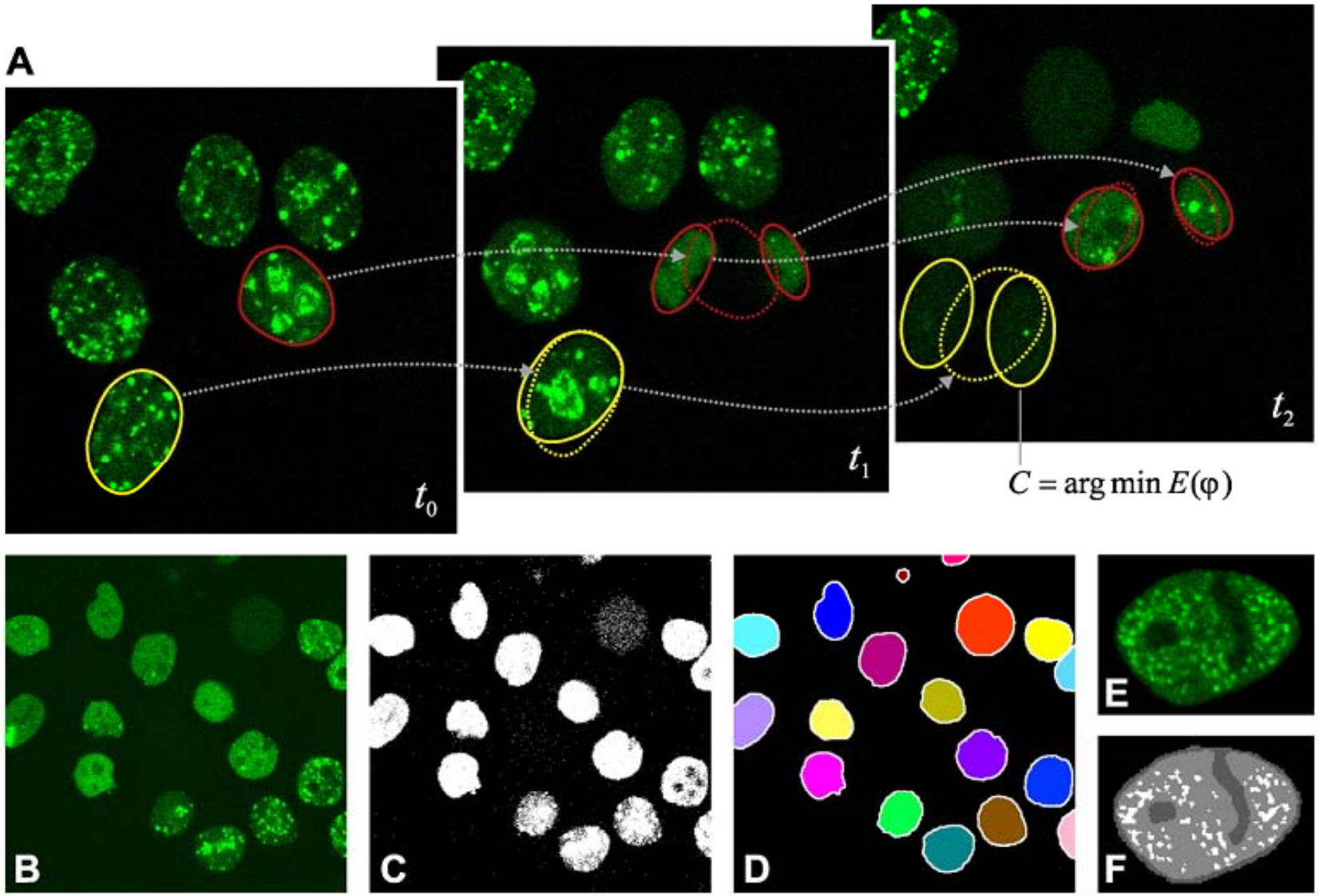



**Fig. 1.** Cell tracking. (A) Three frames from a time-lapse fluorescence microscopy image, illustrating the concept of model-evolution based cell segmentation and association. Cell contours or surfaces can be defined parametrically or as the zero-level of a higher-dimensional function. In each image frame, the final contour of a cell (solid line) is obtained by minimization of an energy functional, typically consisting of image-based and shape-based terms. The initial cell contour (dotted line) for each frame is usually taken to be the final contour from the previous frame. Cell divisions can be detected by monitoring the shape of the contour function during energy minimization. (B) Depending on the type of labeling, the appearance of cells (or in this case their nuclei) may vary greatly, within one frame as well as over time. (C) Thresholding usually results in a very noisy (at low thresholds) or fragmented (at high thresholds) segmentation. (D) Model-based segmentation (in this case using level sets) can yield much more sensible results. Once all cells are tracked, they can be easily extracted individually (E) and geometrically transformed to a reference coordinate-frame for subsequent intracellular analysis (F).

# C e l l Association

- After segmentation, the second step in achieving cell tracking is cell association. This refers to the process of identifying and linking segmented cells from frame to frame in the image sequence to obtain cell trajectories. The simplest approach to accomplish this is to associate each cell in any frame to the spatially nearest cell in the next frame (for example according to centroid position)within a predefined range. However, when dealing with many cells or rapid cell movements, this may easily lead to mismatches.

# Cell Association

Several strategies exist for performing interframe cell association. The simplest is to associate each segmented cell in one frame with the nearest cell in a subsequent frame, where nearest may not only refer to spatial distance between boundary points or centroid positions. It may refer to similarity in terms of average intensity, area or volume, perimeter or surface area, major and minor axis orientation, boundary curvature, angle or velocity smoothness, and other features. Generally, the more features involved, the lower is the risk of ambiguity. However, matching a large number of features may be as restrictive as template matching, since cell shape changes between frames are less easily accommodated. Some applications may not require keeping track of cell shape features, so robust tracking of only cell center position may be achieved by mean-shift processes.

- The concept of template matching, for example, can serve as a basis for image registration between time points. Registration refers to the process of (global or local) alignment of images, using intensity- or geometry-based features.
- In the case of deformable models, cell association can be performed “on the fly”, by using the segmentation results in any frame as initialization for the segmentation process in the next frame. (works well if the population density is not too high)
- gradient-vector flows
- probabilistic schemes

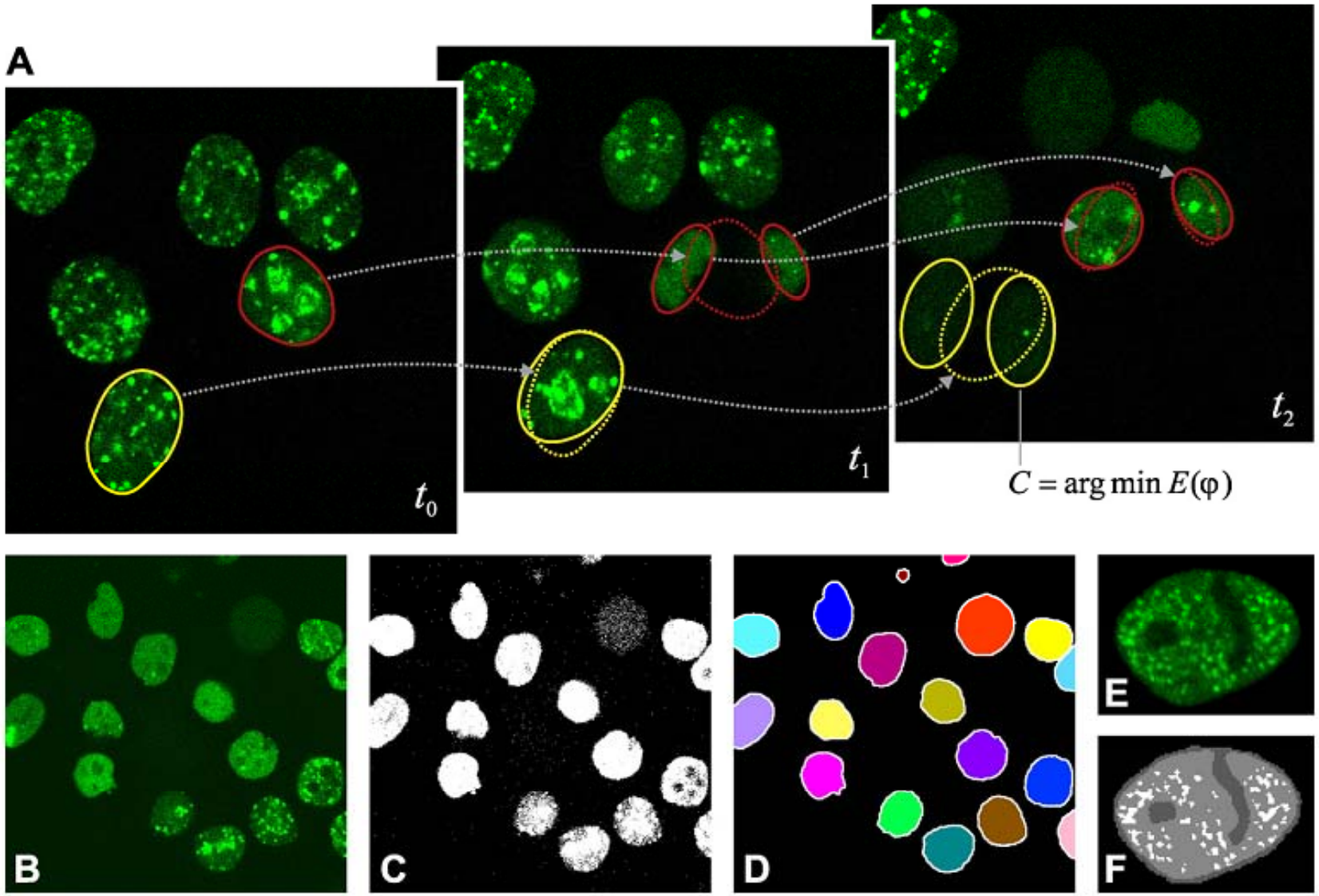



**Fig. 1.** Cell tracking. (A) Three frames from a time-lapse fluorescence microscopy image, illustrating the concept of model-evolution based cell segmentation and association. Cell contours or surfaces can be defined parametrically or as the zero-level of a higher-dimensional function. In each image frame, the final contour of a cell (solid line) is obtained by minimization of an energy functional, typically consisting of image-based and shape-based terms. The initial cell contour (dotted line) for each frame is usually taken to be the final contour from the previous frame. Cell divisions can be detected by monitoring the shape of the contour function during energy minimization. (B) Depending on the type of labeling, the appearance of cells (or in this case their nuclei) may vary greatly, within one frame as well as over time. (C) Thresholding usually results in a very noisy (at low thresholds) or fragmented (at high thresholds) segmentation. (D) Model-based segmentation (in this case using level sets) can yield much more sensible results. Once all cells are tracked, they can be easily extracted individually (E) and geometrically transformed to a reference coordinate-frame for subsequent intracellular analysis (F).

## Particle tracking & association

- Similar to cell tracking & association, the simplest approaches to particle tracking is thresholding and for particle association is to use a nearest-neighbor criterion, based on spatial distance

## Trajectory analysis

- The final stage is the analysis of the trajectories resulting from cell or particle tracking, to confirm or reject predefined hypotheses about object dynamics, or to discover new phenomena.
- Geometry measurements
- Diffusivity measurements
- Velocity measurements

# References

- Matrix
- MATLAB
- Photoshop

# References


K.R. Castleman, *Digital Image Processing*, Prentice-Hall, 1996.

R.C. Gonzalez and R.E. Woods, *Digital Image Processing*, Addison-Wesley, 1992.

A. Bovik, *Handbook of Image & Video Processing*, Academic Press, 2000

J.S. Lim, *Two-Dimensional Signal and Image Processing*, Prentice-Hall, 1990

D. Hanselman and B. Littlefield, *Mastering MATLAB 6: A Comprehensive Tutorial and Reference*, Prentice-Hall, 2001.

*MATLAB Image Processing Toolbox User's Guide*

# References

- **Journals**:
  - IEEE Trans. on Image Processing
  - IEEE Trans. on Medical Imaging
  - Signal Processing, Image Communication (Elsevier Science)
  - IEEE Trans. on Visualization and Computer Graphics
  - IEEE Trans. on Pattern Analysis and Machine Intelligence
  - Pattern Recognition, (Pergamon-Elsevier)
  - Pattern Recognition Letters ( Elsevier)
  - IEEE Trans. on Biomedical Engineering
  - IEEE Trans. on Information Technology in Biomedicine
  - IEEE Trans. on Signal Processing
  - Signal Processing (Elsevier Science)