Image collection and processing

Basic Steps

Image Collection
- Online processing
- Averaging
- Background subtraction

Storage

Processing
- Filters
- Contrast
- Brightness
- Color correction

Feature Extraction
- Thresholding
- Blob separation
- Watershed split

Quantify
- Examples from the real world
Digital Imaging Decisions

Input
Sample

“Cameras”
CCD
Video
PMT
Intensified video
cooled CCD
SEM
TEM
Scanners

Processing

Storage
quantitative analysis
reconstruction
processing

Computers
PCs
Unix workstation (SGI)
Macs

Network:
Novell
NT
NFS
Appleshare

Archive
Tape
CDROM
Opticals etc

Output display

Printers:
Dye sublimation
Solid ink, Ink jet
Lithography
Pictography etc.

Web sites (virtual pub)
CDROM
Slide makers

CBI
What is a digital image

Digital images are composed of pixels

Pixels contain information about intensity and color that can be stored on a computer.
• Each pixel contains information about 1 location in the image
• A sequence of pixels can be combined to make an image.

Digital images are at the heart of all modern microscopies

The rest of this lecture discusses each of these points in detail
What is greyscale?
greyscale gives contrast and intensity for each pixel

• 1bit=2 greyscales (binary)= black and white
• 2bits=2x2=4 greyscales,
• 3bits=2x2x2=8 greyscales etc.
• most cameras 8bit=256 greyscales
• Commonly cooled CCDs are 12 bits=4096 greyscales
Pixels 3: color vs black and white

- Color images are simply 3 black and white images combined, one for each primary color.
- However they are 3 times larger than the black and white image.
- Color is very useful for image processing.

24 bits = 8 bits + 8 bits + 8 bits
Real World Resolution

400 DPI  200 DPI  72 DPI  36 DPI

16 DPI  8 DPI  4 DPI

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This example is 2X2 binning.

Binning adds electron wells from numbers of pixels, only one use of the readout amplifier, therefore less noise.
Types of CCD

**Line transfer**

Most common, least expensive, subject to noise from readout amplifier

**Frame transfer**, expensive, hard to make, fast frame is read off camera as a new frame is collected.
Regular CCDs vs backthinned CCDs

Backthinning increases QE
Detector noise:

- Thermal, or gain
Background subtraction

Subtract background in Fluorescence, removes hot pixels and noise
Background Subtraction

Original/background corrects non-white image

Divide color by itself=white
RGB 0.6|0.9|0.3/ 0.6|0.9|0.3 =1|1|1
Image Averaging

Averaging removes noise. Kalman or proportional average best improvement in image quality is proportional to the square root of the # of frames (4 frames twice as good as 1 frame etc.)

Primary problem is Progressive bleaching and Phototoxicity in Live cell work,
Fast Fourier Transform and noise reduction

- Only useful on repetitive noise, which will generate a Fourier transform

Courtesy Mediacybernetics
Thresholding

• Generally features are extracted by greyscale value.
Feature extraction

Fill holes with dilation

1 2 3

Erosion to separate structures

1 2 3

Use as mask on original to define Splits

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Features can be extracted by size, shape, etc any measurable parameter can be used to classify objects.

Image collected

Threshold to select features

Objects classified by size (more than, less than) size of hole etc in this image object classified by ratio of hole to perimeter.

Take home is that objects can be classified by any mathematically definable parameter.
Use transmitted light image of melanin to extract tumor volume

Fluorescence image of labelled cells in lung

Bright field image of lung bearing tumor

Threshold image to show melanin

Average image (3X3 filter) to remove camera noise and make binary
Invert binary image, such that now melanin is white

Pass binary image through multiple pixel dilates to fill holes

original

Single pixel dilation

Two pixel dilation

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Holes have been filled, however the profile is bigger than the tumor so erode pixels
Use mask to extract NK profiles in tumor count number of cells in field automatically
Video rate vs Cooled CCD

Cosmic Rays or X-Rays

Photons

Silicon Target

HEAT = thermal noise
Removed by cooling CCD

Noise increases with Amplifier Bandwidth
Video rate CCD (30 frames/sec) must read at 10 million pixels/sec
Slow scan cameras work at only 500,000 pixels/sec
Therefore slower and less noisy

Readout amp

Speed related noise

Amplifier Bandwidth

Video rate CCD (30 frames/sec)

10 million pixels/sec

500,000 pixels/sec

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Intensified Devices

Incoming Photons

- Avalance Photodiode

+ Fiber optic cables

Fiber optic cables

Anode Collector

Photo Multiplier Tube

Dynodes

Photocathode

- Avalance Photodiode

+ Fiber optic cables

Fiber optic cables

CCD

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