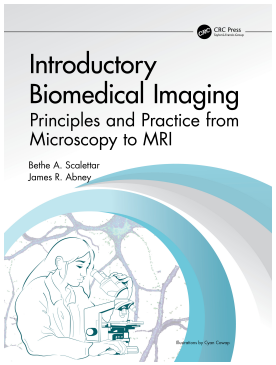
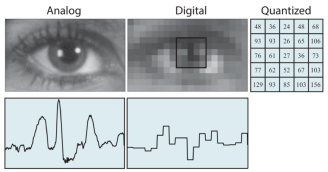


PowerPoint Slides

- Digital Image Processing & Analysis

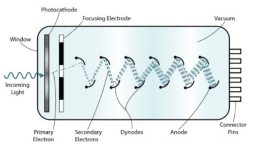
Digital Image Processing and Analysis

- What is a digital image?
  - Useful to compare with analog image/signal
  - Analog signals vary smoothly, like a traditional film-generated photograph or voltage
  - Digital signals vary discretely
- Converting an analog image into a digital format
  - Converts image into 2D array of integers
  - Involves sampling (taking periodic snapshots)
  - Quantization of samples with integers (e.g., 0 to 255)
  - Larger numbers correspond to brighter samples



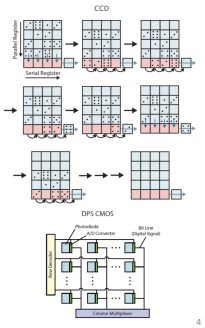
Detectors for Digital Imaging – Part I
Point Detectors

- Photomultiplier tube (PMT) – light into current
  - Very high gain (up to 10^8)
  - High signal-to-noise because dark current is very low
  - Temporal resolution a few hundred picoseconds
  - Low "quantum efficiency," and spectral response is nonuniform
  - https://micro.magnet.fsu.edu/primer/java/digitalimaging/photomultiplier/sideonpmt/index.html
  - https://micro.magnet.fsu.edu/primer/java/digitalimaging/photomultiplier/channel/index.html
- Photodiode – light into current
  - Small point detector
  - Can be assembled into an array that is capable of spatial discrimination
  - Good quantum efficiency
  - Lacks gain



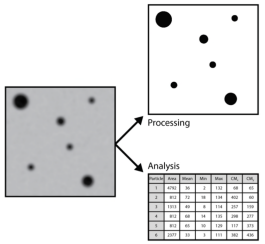
Detectors for Digital Imaging – Part II
Spatially Sensitive Detectors

- Cameras – 2D array of detectors (pixels)
  - Light from object focused onto the camera
  - Photon-induced charge (proportional to the incident intensity) generated in each pixel
  - Charge distribution in detector array generates image of object
- Charge-coupled device (CCD) – the standard for decades
  - Charge is transferred and then converted to a voltage and digitized serially
- Scientific-grade complementary metal-oxide semiconductor (sCMOS) device – the new standard
  - Key features – charge to voltage conversion and, in some cases, digitization occur at each pixel
  - Individual pixels or a subset of pixels can be read out
  - Much faster than CCDs



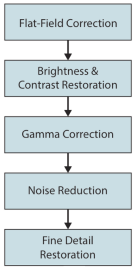
Introduction to Image Processing and Analysis

- Digital images can be visualized, processed, and analyzed on a computer
- Difference between processing and analysis
  - Processing involves modification of an image to produce another image (e.g., grayscale into black & white)
  - Analysis involves obtaining numerical data from images, which may be converted into a visual format
- Possible steps in processing and analysis
  - Image restoration
  - Image enhancement
  - Binary conversion/segmentation
  - Measurement



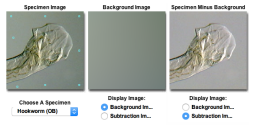
What is Restoration?

- Correcting defects that arise during acquisition from imperfections in the optics, illumination, detector, etc.
- Restoration Strategy



Flat-field Correction & Background Subtraction

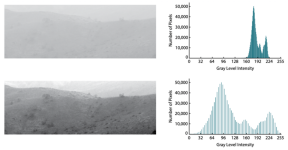
- Flat-field correction
  - Removes degradation from sources like scratches in the optics, detector imperfections, and uneven illumination
  - Important when image is to be quantified
- Background subtraction
  - Visually similar results



Source: https://micro.magnet.fsu.edu/primer/java/digitalimaging/processing/backgroundsubtraction/index.html

Brightness & Contrast Restoration

- Brightness measures image lightness
- Contrast measures grayscale or color variation
- Two tools
  - Histogram = graph of intensities in image on x and number of pixels with intensity on y
  - Input-output lookup table (LUT) = function/rule that transforms an input pixel value into a new output
- Histogram stretching
  - Using histograms and LUTs to enhance contrast
  - Map image minimum into zero and image maximum into 255 (for 8-bit) and ramp linearly in between
  - https://micro.magnet.fsu.edu/primer/java/digitalimaging/processing/histogramstretching/index.html

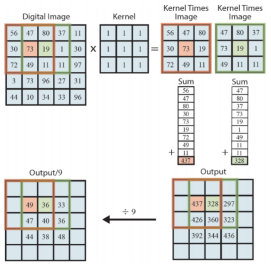


Gamma Adjustment

- Non-linear (exponential) adjustment of histogram to facilitate simultaneous visualization of both bright and dim features
  - For 8-bit image $I_{new} = 255*(I_{orig}/255)^{\gamma}$
  - $\gamma < 1$ enhances differences among dimmer pixels
  - $\gamma > 1$ enhances differences among brighter pixels
  - https://micro.magnet.fsu.edu/primer/java/digitalimaging/processing/gamma/index.html

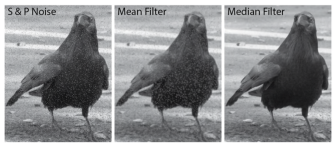
Noise Reduction – Part I

- Noise is a random signal superimposed on the true signal
- Reduction achieved using filters
- Spatial convolution filters
  - Multi-pixel operation → output contains contributions from several neighbors
  - Example – smoothing with mean box filter
  - Overlay kernel containing "ones" on center pixel, multiply, add, and replace center by result
  - http://micro.magnet.fsu.edu/primer/java/digitalimaging/processing/kernelmaskoperation/
  - Mean reduces high-frequency noise but also blurs image
  - https://micro.magnet.fsu.edu/primer/java/digitalimaging/processing/spatialaveraging/index.html
  - Gaussian gives less less weight to distant neighbors – reduces noise but with less blur



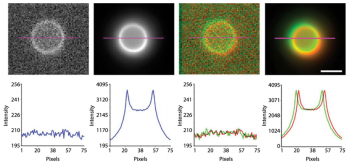
Noise Reduction – Part II

- Median filter
  - Box around pixel, sort, and replace pixel by median
  - Deals effectively with dramatic noise and better at preserving fine detail than convolutions
  - Computationally intensive
  - https://micro.magnet.fsu.edu/primer/java/digitalimaging/processing/medianfilter/index.html



Noise and Resolution

- Diffraction and sampling can affect resolution
- Noise also can affect resolution
  - Noise often causes resolution to be much lower than the diffraction limit
  - Image noise quantified using the signal-to-noise ratio

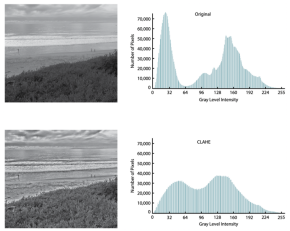


Fine Detail Restoration

- Enhance detail
  - Example = edges
- Unsharp filter
  - Generate an "unsharp" (smoothed) version of image (e.g., using mean filter)
  - Subtract smoothed from image to remove larger-scale detail and produce an image retaining finer detail
  - Add a portion of "edge" image to original to produce sharpened, edge-enhanced image
  - https://micro.magnet.fsu.edu/primer/java/digitalimaging/processing/unsharpmask/index.html

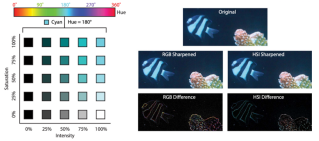
Image Enhancement

- A more subjective process directed at altering an image to influence impact on the observer
- One example – local contrast enhancement
  - One popular method, used by Fiji, is contrast limited adaptive histogram equalization (CLAHE)
  - Effect shown at right



Color Processing and Color Coordinates

- Color processing
  - Smoothing, sharpening, etc.
- Hue, saturation, intensity (HSI) better than RGB for color processing
  - Hue = dominant λ
    Saturation = color purity
    Intensity = brightness
  - HSI decouples brightness from color
  - Convert to HSI and process just intensity channel to avoid introducing color shifts



Binary Conversion & Image Segmentation

- Binary Conversion
  - Converting grayscale or color image to a 1-bit (black and white) image
  - Used to segment an image into objects of interest and uninteresting "background"
  - Segmentation widely used
- Histogram-based thresholding
  - Simple case – histogram has two peaks for objects and background
  - Set threshold in valley so objects above are white and objects below are black

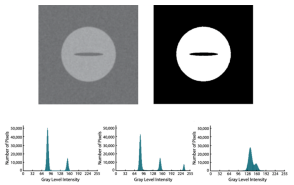


Image Analysis (Measurement) – One Important Example –

- Single particle tracking (SPT)
  - Track individual vesicles manually using Fiji
  - Determine x/y coordinates as a function of time
  - Determine trajectories, speeds of motor-directed transport, and diffusion coefficients