Digital Imaging in Microscopy

Maize embryo sac
Acriflavine staining
Hand Drawing
The Camera Lucida
Film Photography

Film plane at focal plane of lens

Photo tube

Projection Lens

• 4x5" film
• 35 mm film

• Black & White film
• Color film
Video Imaging — Analog Technology

![Diagram of video imaging circuit]

- **First Pass**
- **Second Pass**

- **Signal Current**
- **Video Signal Voltage**
- **Target Voltage Supply**

- **Target Electrode**
- **Photoconductive Layer**
- **Electron Beam**
- **Cathode**
- **Filament**

- **525 lines**
- **Interlacing**

- **ΔT = 1/60 s (fields)**
- **Full frame image = 1/30s**
TV Standards

National Television Standards Committee — NTSC, PAL, SECAM

- 525 TV lines
- 60 Hz field rate

Realized resolution = 350 lines
Digital Imaging using Analog Cameras

Analog video → Frame grabber → Resultant digital image is 640x480 pixels

analog-to-digital
Increasing Light Sensitivity of Video Detectors

Silicon Intensified Target (SIT) Camera

Vidicon Image intensifier
Solid State Detectors: The CCD

Nikon D100 CCD

Cannon digital camcorder CCD
Solid State image detectors

Typical CCD Chip
Specification (no./size of pixels, etc.) varies according to chip model

- 1024 elements (columns)
- 256 elements (rows)
- Image Area
- Shift Register
- Amplifier
- Output Node*
- 26 square micron element or pixel

*Charge that is output from the shift register may be summed either on- or off-chip, depending on the chip model.
Front-Illuminated CCD

Photons travel through the silicon gate structure
CCD size

Traditionally measured as a fraction

CCD chips come in many sizes and bit-depths

http://www.astrosurf.com/re/chip.html
Resolution and Sampling

Nyquist sampling

\[ 1/T > 2f \]

The rate of sampling must be at least twice the frequency of the resolvable desired structure (signal): “Nyquist Rate”
Matching microscope resolution and CCD pixel size

1. Determine microscope resolution

\[ d = \frac{0.61\lambda}{NA} \]

where \( NA \) is the average of the objective and condenser.

2. Match the microscope resolution with (a minimum) of two pixels on the CCD

\[ M \cdot \frac{0.61\lambda}{NA} = 2.0p \]

where \( M \) = the objective magnification; \( p \) = pixel size

\[ M = \frac{2p}{0.61\lambda/NA} \]

e.g., CCD with 6.8μ pixels and green light (500nm) illumination:

\[ M = \frac{2 \cdot 6.8\mu}{(0.61 \cdot 0.5\mu/1.3)} \]

\[ M = 58 \]

Thus, a 60x, 1.3 N.A. microscope objective provides a diffraction-limited image for the KAF1401E CCD camera.
Bit Depth and Dynamic Range
Greater Bit-Depth and Dynamic Range Records More Information

8-bit

12-bit
Quantum Efficiency and Spectral Response

QE vs. Impinging wavelength

QE = photons/electrons

Native

Coated
Increasing Quantum Efficiency (QE) of CCD

Backside-illuminated CCD

Etching uniformly thins a CCD to a thickness of approximately 10 µm. The image is focused on the backside of the CCD register (where there is no gate structure). Thinned CCDs exhibit high sensitivity to light from the soft x-ray to the near-infrared regions of the spectrum.
Increasing Signal Readout Rate

Serial Clocks
Parallel Clocks
Output Amp

Interline Transfer CCD
Dark Current and CCD Temperature

Single-Stage Peltier Cooling
-35°C
Optronics DEI 450
Two-Stage Cooling

Heat from Peltier drawn off by recirculating liquid

Photometrics CH350 digital camera
Color Image Capture

Epifluorescence microscope with color image capture
Color Imaging — 3 CCDs

Dichroics  Filters  CCDs

3  2  1

Video (640x480) Technology
Color Imaging — 1 CCD

0.6 MPix (1995)
1/3” Sony frame transfer CCD

5.5 MPix (2003)
2/3” Sony ICX282 Progressive Scan Interline CCD
Example Digital Cameras
Photometrics Quantix
KAF 1401E

Frame readout CCD

1035x1317 pixels
6.8\(\mu\)m x 6.8\(\mu\)m pixels
12 bit pixel depth
5MHz readout
Two-Stage cooling to -50\(^\circ\)C
Photometrics CoolSnap HQ

**Interline transfer CCD**

- 1040x1392 pixels
- 12-bit pixel depth
- 6.45µm x 6.45µm pixels
- 20MHz readout
- Single-stage cooling to -30°C
QImaging
MicroPublisher

Sony ICX282 Progressive Scan Interline CCD, Color
2560 x 1920
3.4µm x 3.4µm
10-bit
20, 10, 5, 2.5MHz
Peltier thermoelectric cooling to 10C below ambient