Nanoscopy

Marcel, Wolfgang

Biomolecular Photonics, Bielefeld University

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1. Light detection
   - Semiconductors: CCDs and CMOS
   - Analog / digital conversion

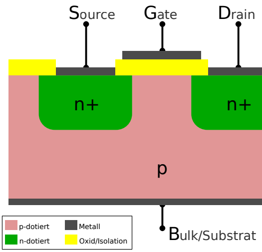
2. Camera properties
   - Linearity and gain
   - Quantum efficiency and noise
   - Sampling precision / "bit depth"

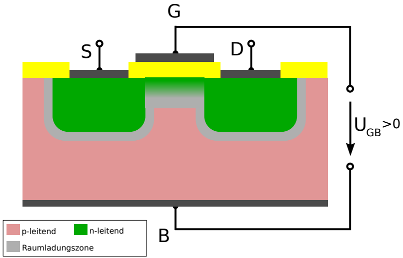
3. Digital image processing
   - Data formats
   - Human vision
   - Gamma correction
   - Processing and displaying images
   - Storing images / file formats
   - From measurement to publication

Light detection: Semiconductor-based cameras

MOSFET

- MOSFET / field-effect transistor
- Charge on gate sets conductivity
- No current through gate required: Measuring gate charge as voltage

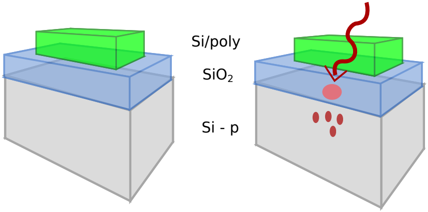




WikiMedia: MOSFET, MOSFET with channel

Pixels

Photosensitiv semi-conductor pixel

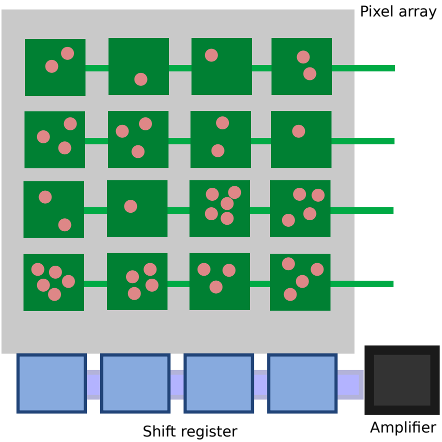


- Top layer: Conductive, transparent material, e.g. poly-crystalline silicon
- Middle layer: Insulating material, e.g. silicon dioxide
- Bottom layer: positively doped semiconductor, again silicon

- Top electrode charged negatively, creates depletion zone
- Incoming photon creates electron/depletion pair, electrons accumulate
- Question: How to measure the amount of collected electrons?

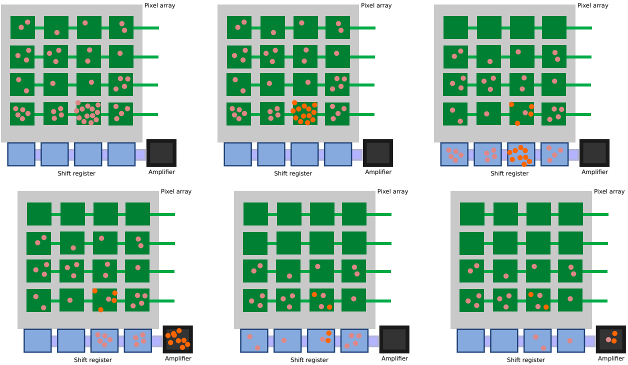
CCDs

- *Charge coupled device:* Named after the working principle
- Invented late 1960, Nobel Prize 2009
- After exposure, charge is moved down pixel by pixel.
- Reaching end of the sensor, charge is moved right into a shift register
- Note: charges are moved by applying a field, not by creating a circut.



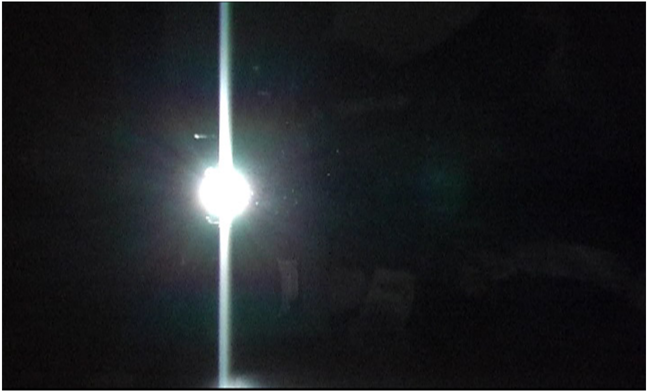
CCDs: Readout

CCDs: Readout of overexposed pixels



Problem of CCDs: Overexposed pixels can influence a complete vertical readout line, as their charge is not completely transferred.

CCD: Overexposure and vertical blooming



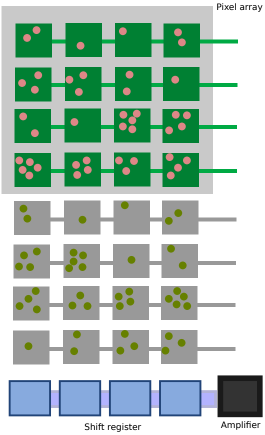
Blooming of overexposed pixel and along readout. Wikipedia

Effect of this problem: Vertically smeared lines around overexposed pixels.

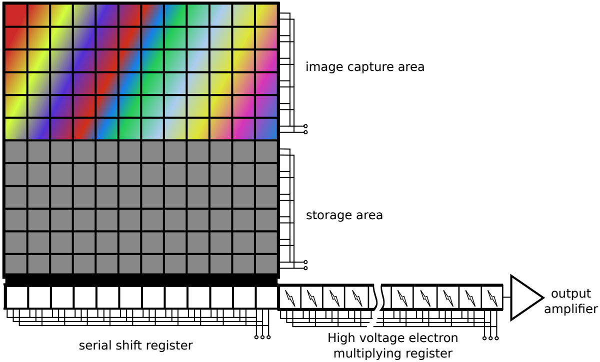
CCDs: Types / faster readout modes

Speed of CCDs:

- Each pixel has to pass through the same electronics, makes it slow
- Readout while exposure can give artifacts
- Shift each line of even a full frame to a non-light sensitive area
- Parallelize read-out



em-CCD: Precise readout through pre-amplification



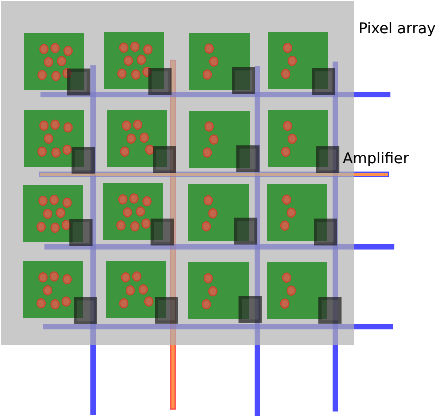
emCCD working principly (Wikimedia)

Advantage of CCDs: One (or few) amplifiers for all pixels.

- Build an electron-multiplying pre-amplifier
- Pre-amplification gives low noise, high sensitivity
- Sensor content is shifted into storage, allows read-out during next exposure
- Cameras are rather expensive

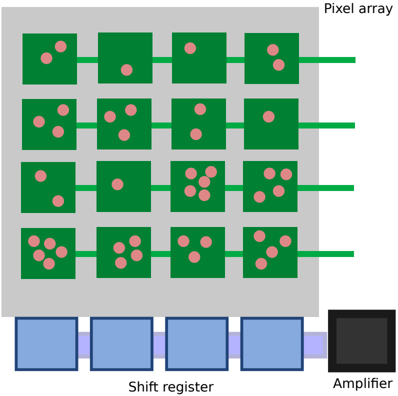
CMOS: No more need for shifting electrons

- *Complementary metal-oxide-semiconductor* named after how they are produced
- **Active pixel sensor** Arguably the more correct term
- Place the amplifier (transistor/diode combinations) with each pixel
- Simple switching matrix, only measure a voltage: fast
- With CMOS process: cheap(er) to produce
- *Scientific CMOS*: nice marketing term for CMOS chips optimized for scientific use.



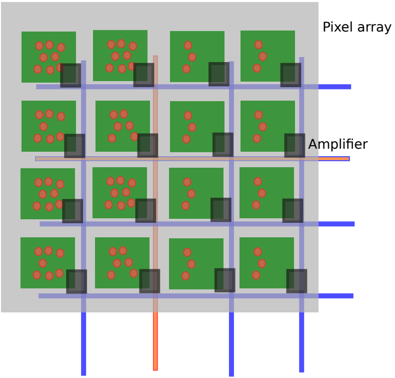
Comparison: CCDs and CMOS

CCD:



Charges are moved to one (or a few) amplifiers

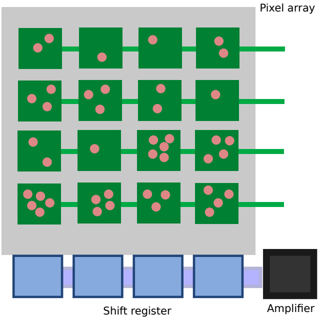
CMOS:



Dedicated MOSFET to convert charge to conductivity for each pixel

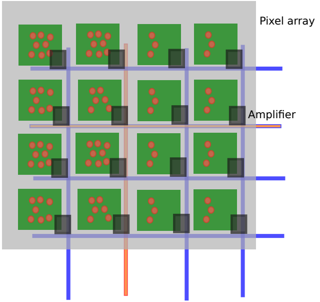
Pros and cons: CCDs and CMOS

CCD:



- Allow for electron-multiplying amplifiers
- Allow for pixel-binning
- Same amplifier characteristic for each pixel

CMOS:



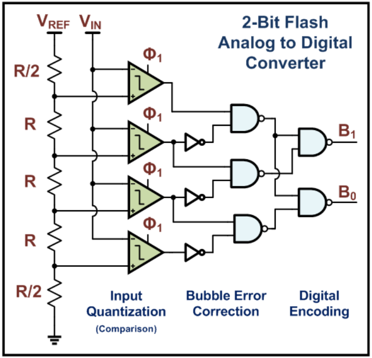
- Cheap(er) to produce
- Fast, parallel read-out
- Electronic shutter

Digital image processing

So far, the CCD/CMOS signal is still analog (electron count / voltage).
How to obtain a digital signal?
How is that signal linked to the measurement?
How to display / represent that signal?

Analog-digital conversion

- CCD (after amplifier) and CMOS provide a voltage (analog) proportional to the amount of photons received.
- **ADC**s (analog-digital converters) are used to convert that voltage into a digital signal.
- Result (usually) a binary number N, where $\frac{N}{N_{\text{max}}} = \frac{V}{V_{\text{ref.}}}$ and $N_{\text{max}} = 2^b$ given by the number of bits b, typ. $b = 8, 12, 16, 24, \ldots$.
- Is the ADC linear? Does is introduce measurement errors?



Flash ADC (Wikimedia)

Camera linearity and gain

Photon count $P \rightarrow$ Electron Charge $\rightarrow$ Voltage $\rightarrow$ Binary Integer Number N

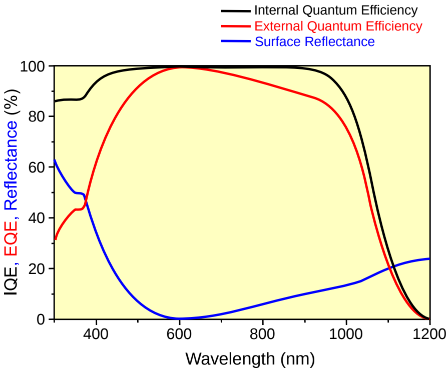
Considerations for microscopy:

- $P \rightarrow N$ is of interest, intermediate steps not so much
- $P \rightarrow N$ is linear, so $P \approx a \cdot N$, ideally with some known a.
- a is called **Gain**.
  Depending on the camera, it can be influenced, especially for emCCDs.
  For CMOS, is will vary by pixel.
- If $P \rightarrow N$ is *not* linear (cheap/non-scientific camera), the function should at least be known.
  If its not known, it can of course be measured.

Camera quantum efficiency and noise

Photon count P → Electron Charge → Voltage → Binary Integer Number N

- **Quantum efficiency:** Ratio of photons converted into stored electrons. Very wavelength-dependent.
- **Noise:** Uncertainty in photon → electron conversion. Electrons tunneling in and out of pixels.
- **Also noise:** Electron → voltage conversion. Depends on ADC type. Greatly reduced by electron multiplying stages.



Ideal QE for silicon with anti-reflective coating. (Wikimedia Link)

Camera bit depth

Photon count P → Electron Charge → Voltage → Binary Integer Number N

- ADC output is an integer N, with some $N_{max} = 2^b$, where b is the **bit depth**.
- Given a gain a, $P_{max} = a \cdot N_{max}$ limits the maximum number of photons.
- In practice: Fix P_{max}, which links bit depth and gain. Now, the bit depth sets the pixel count resolution.
- Ideal camera: P_{max} is higher than the pixel photon limit at a gain of $a = 1$.
- **Bit depth** is the technical inspired term, converts nicely to a **dynamic range**. Or, for photography, directly to aperture **stops**.

Typical camera bit ranges

n	2^n	$\approx$ dB
8	256	24
12	4096	36
14	16384	42
15	32786	45
16	65536	48

Digital image processing

Digital image representation

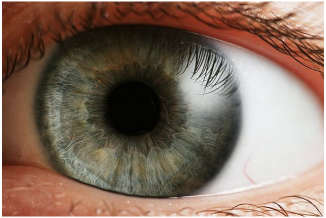
- Typical image file format: 8 bit depth
- That means 256 intensity variations from black to white
- Surely, the eye can distinguish more than 256 gray values?
- An intensity is a measurement, but are images really measurements?

FF	FE	FD	FC	FB	FA	F9	F8	F7	F6	F5	F4	F3	F2	F1	F0
EF	EE	ED	EC	EB	EA	E9	E8	E7	E6	E5	E4	E3	E2	E1	E0
DF	DE	DD	DC	DB	DA	D9	D8	D7	D6	D5	D4	D3	D2	D1	D0
CF	CE	CD	CC	CB	CA	C9	C8	C7	C6	C5	C4	C3	C2	C1	C0
BF	BE	BD	BC	BB	BA	B9	B8	B7	B6	B5	B4	B3	B2	B1	B0
AF	AE	AD	AC	AB	AA	A9	A8	A7	A6	A5	A4	A3	A2	A1	A0
9F	9E	9D	9C	9B	9A	99	98	97	96	95	94	93	92	91	90
8F	8E	8D	8C	8B	8A	89	88	87	86	85	84	83	82	81	80
7F	7E	7D	7C	7B	7A	79	78	77	76	75	74	73	72	71	70
6F	6E	6D	6C	6B	6A	69	68	67	66	65	64	63	62	61	60
5F	5E	5D	5C	5B	5A	59	58	57	56	55	54	53	52	51	50
4F	4E	4D	4C	4B	4A	49	48	47	46	45	44	43	42	41	40
3F	3E	3D	3C	3B	3A	39	38	37	36	35	34	33	32	31	30
2F	2E	2D	2C	2B	2A	29	28	27	26	25	24	23	22	21	20
1F	1E	1D	1C	1B	1A	19	18	17	16	15	14	13	12	11	10
0F	0E	0D	0C	0B	0A	09	08	07	06	05	04	03	02	01	00

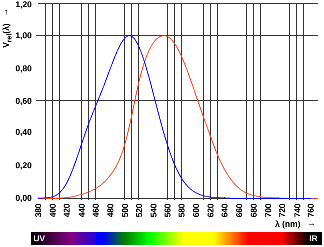
All 256 8-bit gray scale values, with hexadecimal numbering. (Wikipedia)

The human eye

- Human eye has a contrast range of approx. 2^{20}, over all 2^{24} (adaption via iris).
- However, vision is highly non-linear: *You can distinguish small variances in luminescence on a dark background, but not on a bright background.*
- Left: 0% Background, 10% Text
  Right: 50% Background, 60% Text

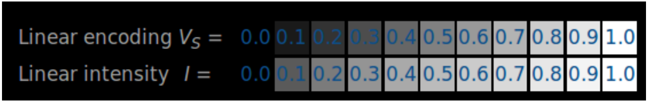


A human eye (Wikimedia)



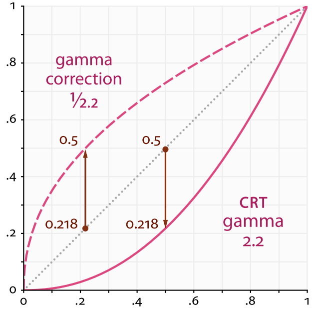
Human eye wavelength (Wikimedia)

Gamma values / Gamma correction



Wikipedia: Gamma Correction / Power law

- Map intensities I and stored values V to a range $[0 \ldots 1]$.
- Link intensity I and stored values V via a power law: $I = V^\gamma$
- A common value (analog TV, standard PC hardware, ...) is $\gamma = 2.2$. It is based on cheap(er) CRT TV design.
- More involved systems (color-calibrated formats, e.g. sRGB) exist.
- This mimics the eye's response well enough for 8-bit storage.

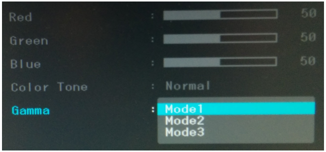


Gamma function graph (Wikimedia)

Images on consumer hardware

Taking and viewing a typical digital photo:
Digital Camera Chip → Image processing → JPEG, 8bit, often sRGB
Facebook / Picassa (auto-awesome!) / SD-Card
PC / Phone / Tablet / TV → 8bit display link (LVDS, HDMI, VGA) → Display driver

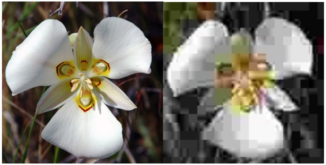
- In 2014: Processing chain will involve 8bit conversions
- Ideal world: Every device knows and applies a correct γ / color space profile.
- Enthusiasts / professionals can get close: Color space management, calibrated equipment, 16 bit raw formats. This needs knowledge and (some) equipment.
- You cannot rely on it!



Typical γ-control on a consumer-grade monitor

Storing images / Image file formats

- Photo/JPEG, Audio/MP3, Video/H264: Highly optimized and compressed for human perception
- PNG: Portable network graphics
  - Lossless compression
  - Stores γ and allows 16bit gray-scale
  - Fully specified, free, open...
  - Well integrated: Web browsers, operating systems, PDF, LaTeX
- TIFF: Tagged image file format
  - Actually a container format (think zip file)
  - Can store collections ("stacks") of images
  - Can store various meta-data
  - Complex to implement, lots of optional features
  - Goto (raw) measurement storage format
- Note: Image file formats used for storing measurements



JPEG Compression, Wikimedia (1, 2)

JPEG vs. PNG, Wikimedia,

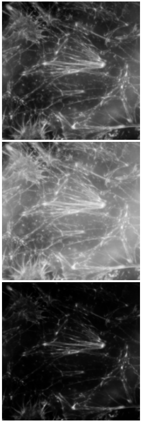
Measurement to image

12-16 bit lin. measurement $\rightarrow$ (documented/limited) processing $\rightarrow$ 8 bit γ-encoded "bitmap"

$$I = V^{\gamma}$$

Convert from a linear "number of photons" to a gray-scale image:

- What represents "black"? Background subtraction?
- What represents "white"? Compensation for overexposed pixels?
- What is the output γ mapping? Linear ($\gamma = 1$) needs more than 8 bit, other non-standard ($\gamma \approx 2.2$) values can be tricky.
- Any further "improvements"?
- The average image processing software displays pixel values in V, not I.
- **Document what is done!**

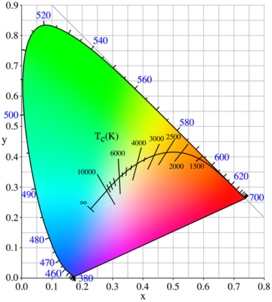


Measurement to image: in color

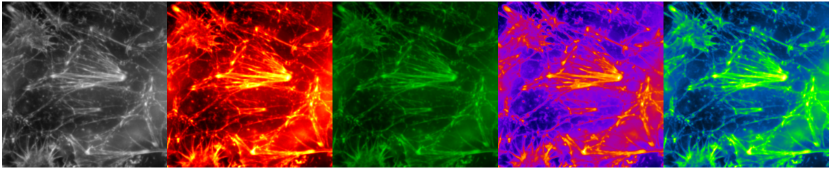
12-16 bit lin. measurement → (documented/limited) processing → 8 bit color (RGB?) image

$$I = V^{\gamma}$$

- All that has been said for gray-scale
- How to map V-values to color?
  - by wavelength
  - by a lookup table
- **Document the colors used!**



Gamut and color temperature (Wikimedia)



Do not rely on software/hardware you have no control of

Creating and viewing images in a typical scientific publications:

12-16 bit lin. measurement → (documented/limited) processing → 8 bit γ-encoded "bitmap"
Publication as PDF, with embedded images
Systems you do not know → Display / Print-out

For digital publication, if possible:

- Do not rely on optional / exotic features
- Do not rely on perfect contrast, gray-scale reproduction (think recycling paper)
- Expect gray-scale print-outs
- Expect color-blind people reading the publication

For seminar / conference talks, if possible:

- Except non-ideal contrast on the projector
- Often one computer for all speakers: No special software, rely on standard formats
- Have a basic PDF version of the talk