

Advanced light microscopy

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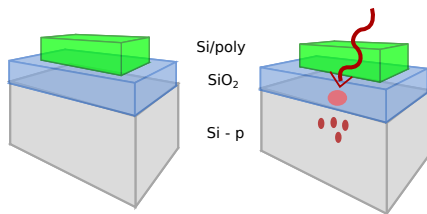
WS 2014

1 Part 2: Digital Image Sensors

2 Camera properties

3 Digital image processing

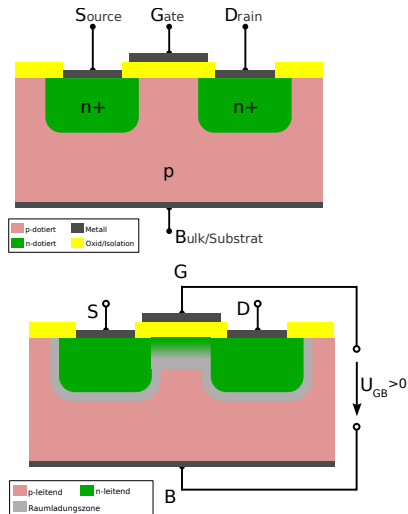
Photosensitiv semi-conductor pixel



- 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?

MOSFET

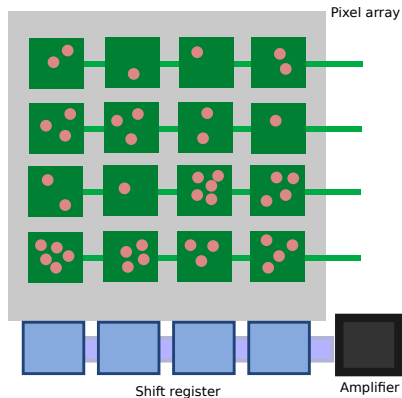
- MOSFET / field-effect transistor
- Charge on gate sets conductivity
- No current through gate required:
Measuring gate charge as voltage



WikiMedia: [MOSFET](#), [MOSFET with channel](#)

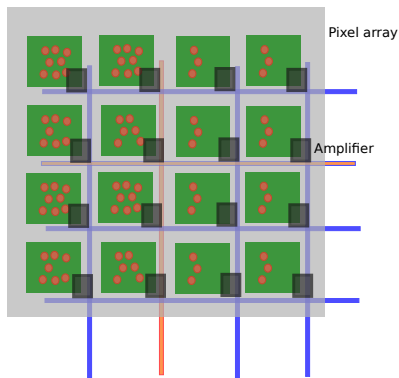
Comparison: CCDs and CMOS

CCD:



Charges are moved to one (or a few) amplifiers

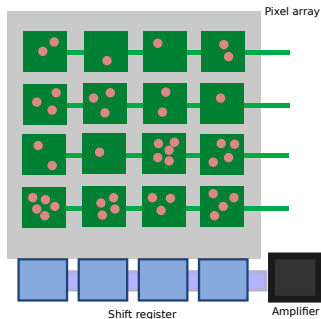
CMOS:



Dedicated MOSFET to convert charge to conductivity for each pixel

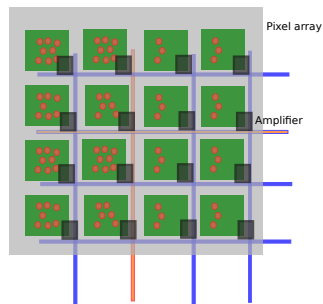
Pros and cons: CCDs and CMOS

CCD:



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

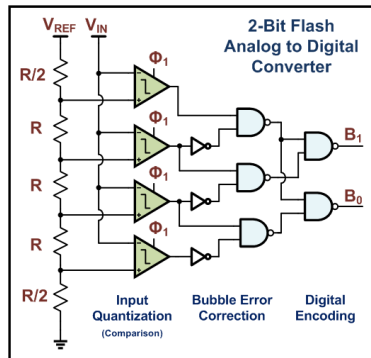
CMOS:



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

Analog-digital conversion

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



Flash ADC (Wikimedia)

Camera (and image) properties

Camera linearity and gain

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

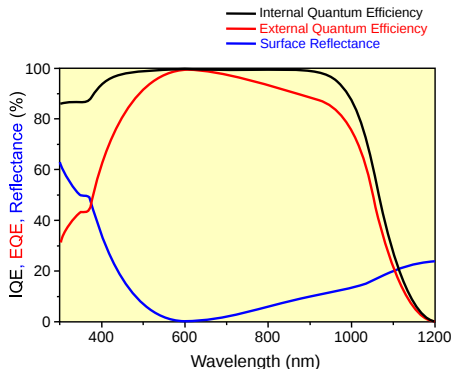
Considerations for microscopy:

- $P \rightarrow N$ 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, it 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 $P \rightarrow$ Electron Charge $\rightarrow$ Voltage $\rightarrow$ Binary Integer Number N

- **Quantum efficiency:** Ratio of photons converted into stored electrons. Very wavelength-dependent.
- **Noise:** Uncertainty in photon $\rightarrow$ electron conversion. Electrons tunneling in and out of pixels.
- **Also noise:** Electron $\rightarrow$ 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 $P \rightarrow$ Electron Charge $\rightarrow$ Voltage $\rightarrow$ 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

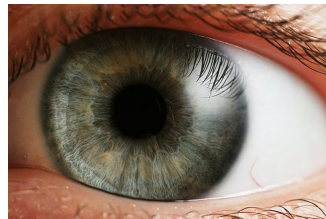
- Typical image file format: 8 bit depth
- That means 256 intensity variations from black to white
- Surely, the eye can distinguish more than 256 gray values?
- An intensity is a measurement, but are images really measurements?

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

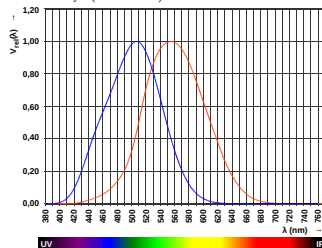
All 256 8-bit gray scale values, with hexadecimal numbering. ([Wikipedia](#))

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)



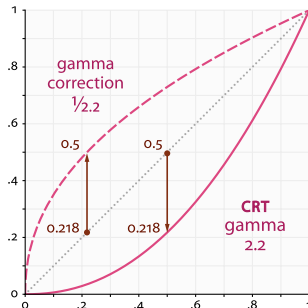
Human eye wavelength (Wikimedia)

Gamma values / Gamma correction

Linear encoding V_S =	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Linear intensity I =	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0

Wikipedia: Gamma Correction / Power law

- Map intensities I and stored values V to a range $[0 \dots 1]$.
- 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 (Wikipedia)

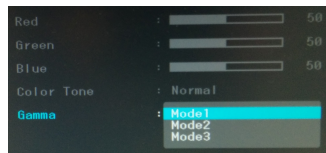
Taking and viewing a typical digital photo:

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

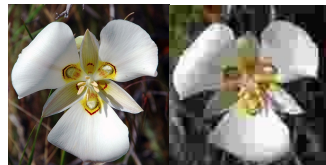
- In 2014: Processing chain will involve 8bit conversions
- Ideal world: Every device knows and applies a correct γ / color space profile.
- Enthusiasts / professionals can get close: Color space management, calibrated equipment, 16 bit raw formats. This needs knowledge and (some) equipment.
- You cannot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



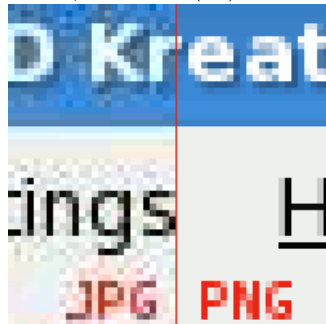
Typical γ -control on a consumer-grade monitor

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, $\text{\LaTeX}$
- 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)



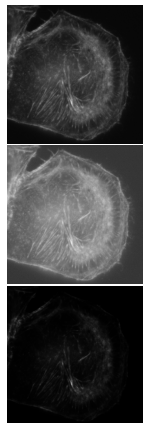
JPEG vs. PNG, Wikimedia,

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!**



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 $\rightarrow$ (documented/limited) processing $\rightarrow$ 8 bit γ -encoded "bitmap"
Publication as PDF, with embedded images

Systems you do not know $\rightarrow$ 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