3 Illumination and detection

MOL/BPY504B

Roadmap

- Illumination sources, from lamps to lasers
- Digital imaging
- Single pixel devices: PMT and APD
- Camera technologies: CCD and CMOS
- Microscope Stages

Illumination subsystem

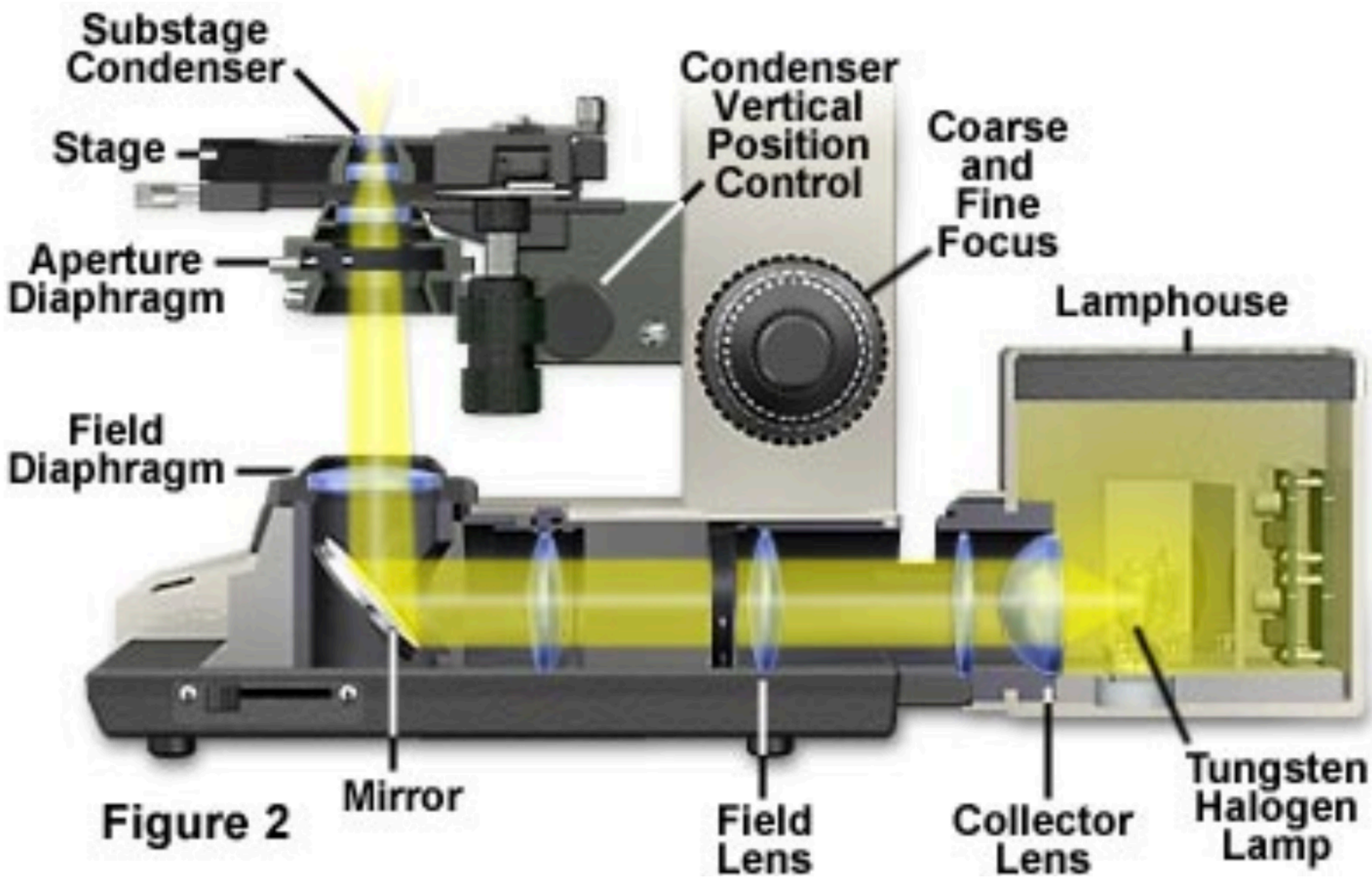


Figure 2

Mercury arc lamps

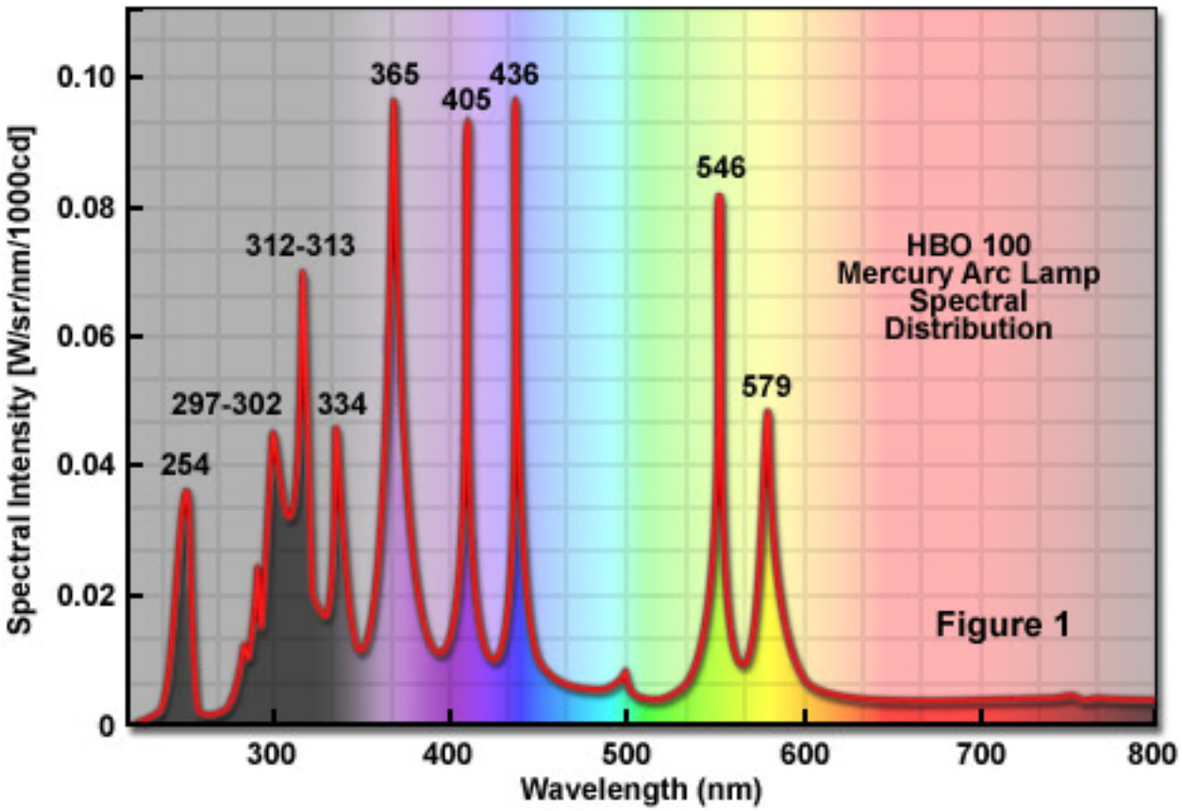
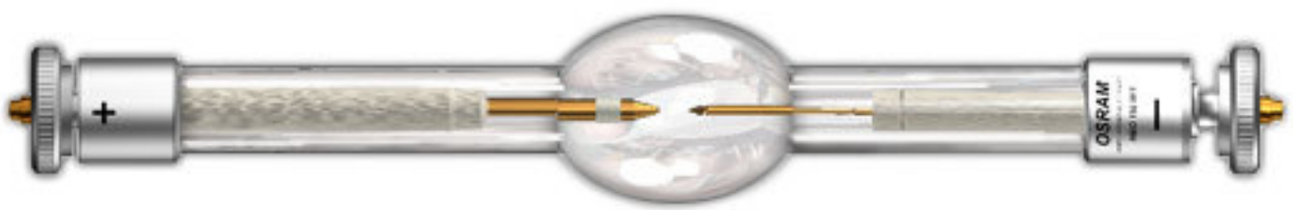


Figure 1



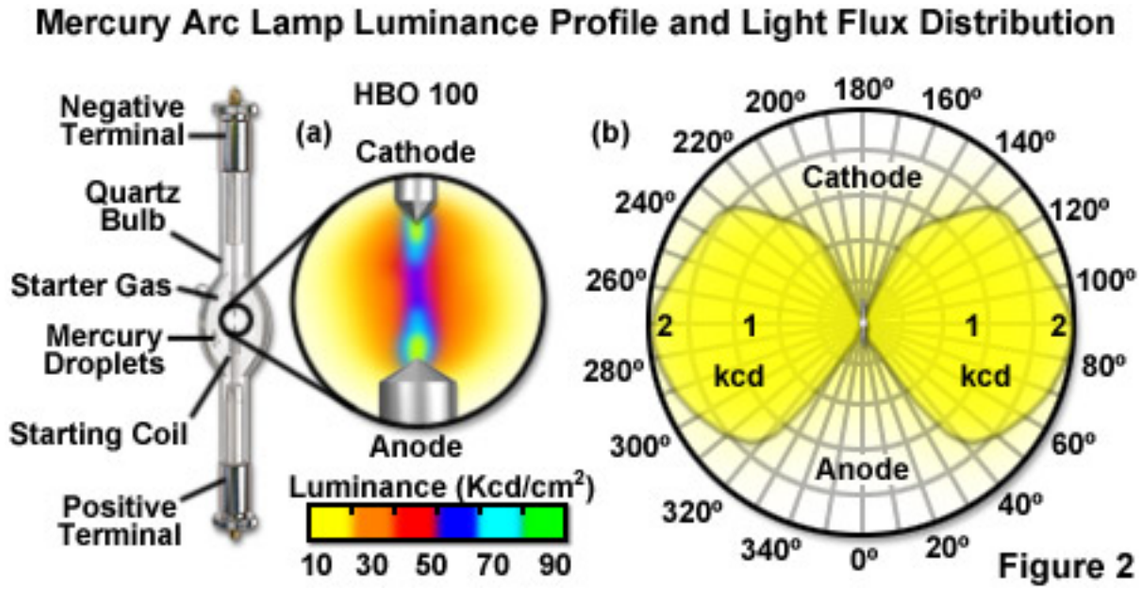


Figure 2

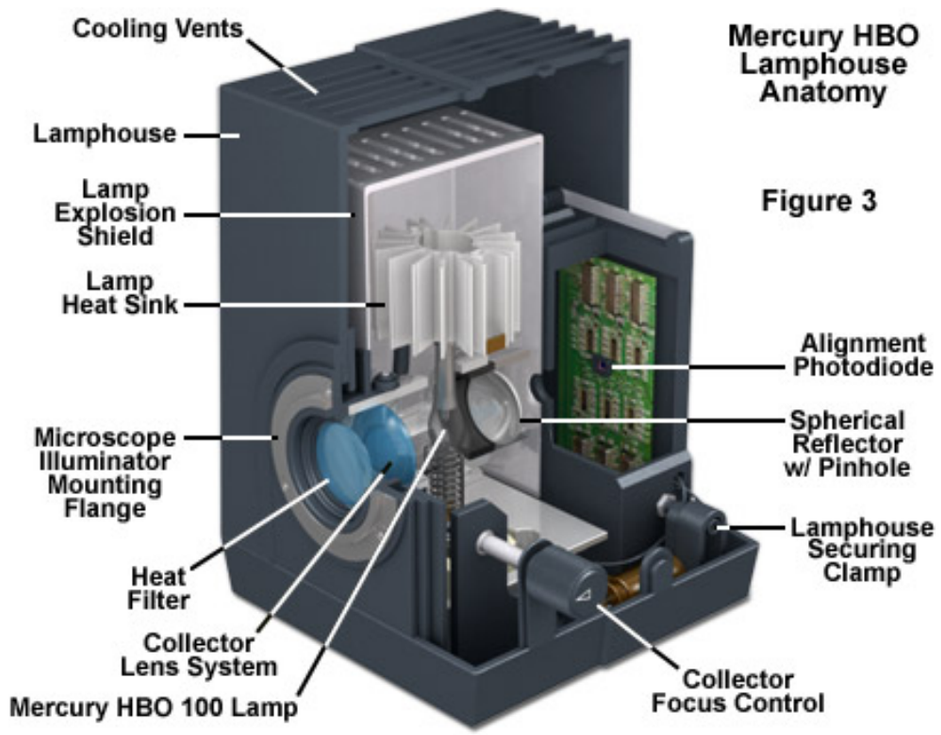
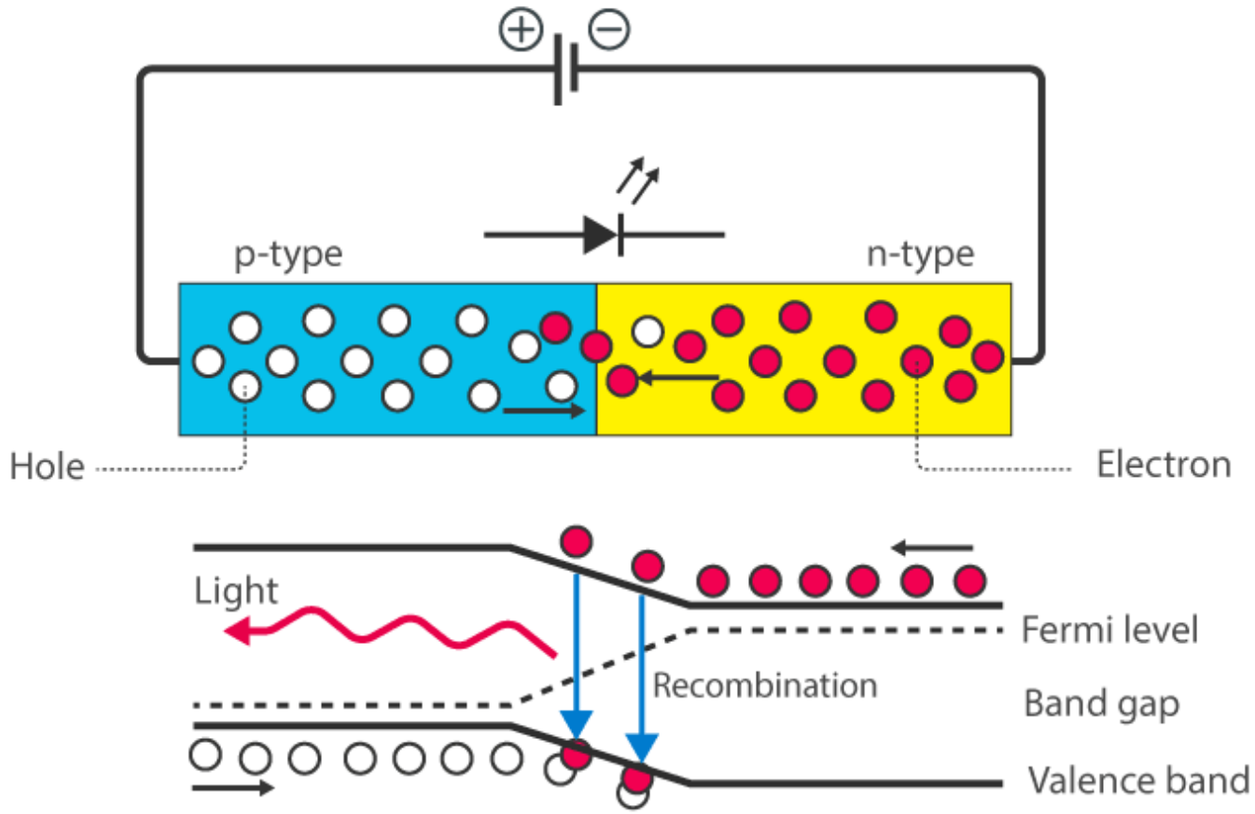
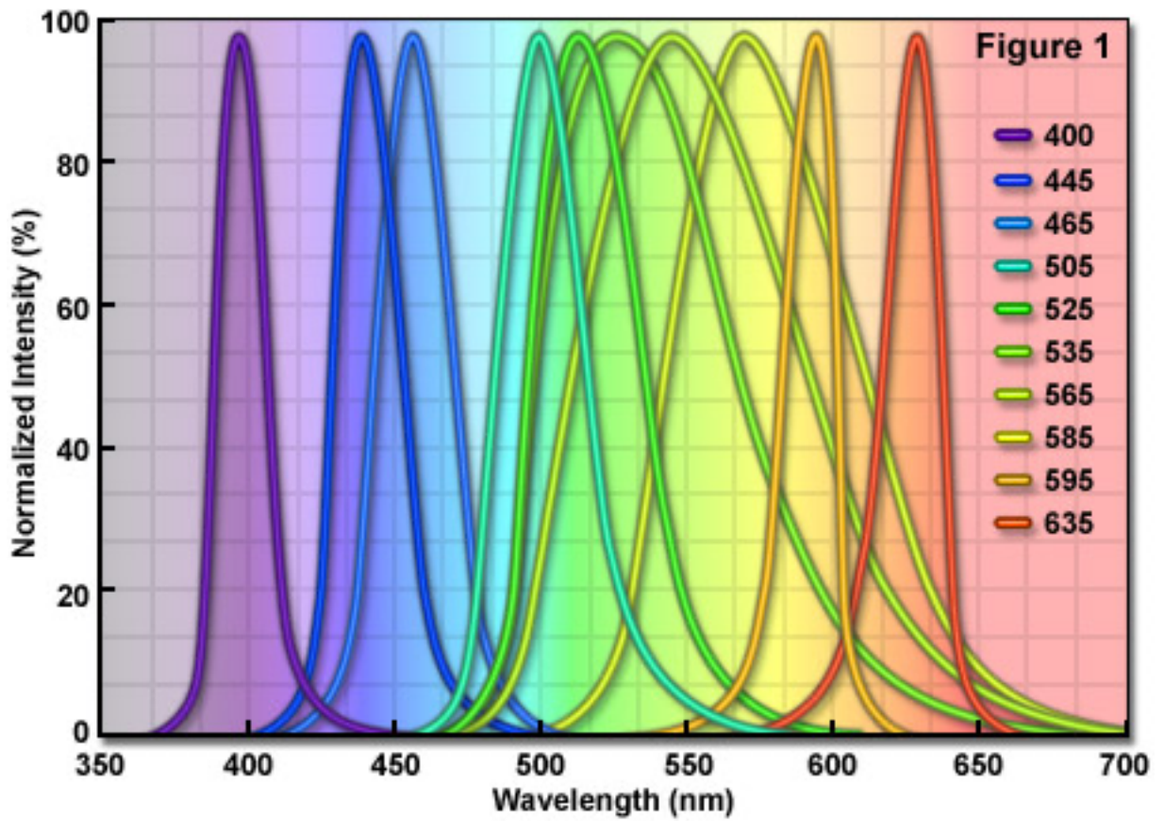


Figure 3

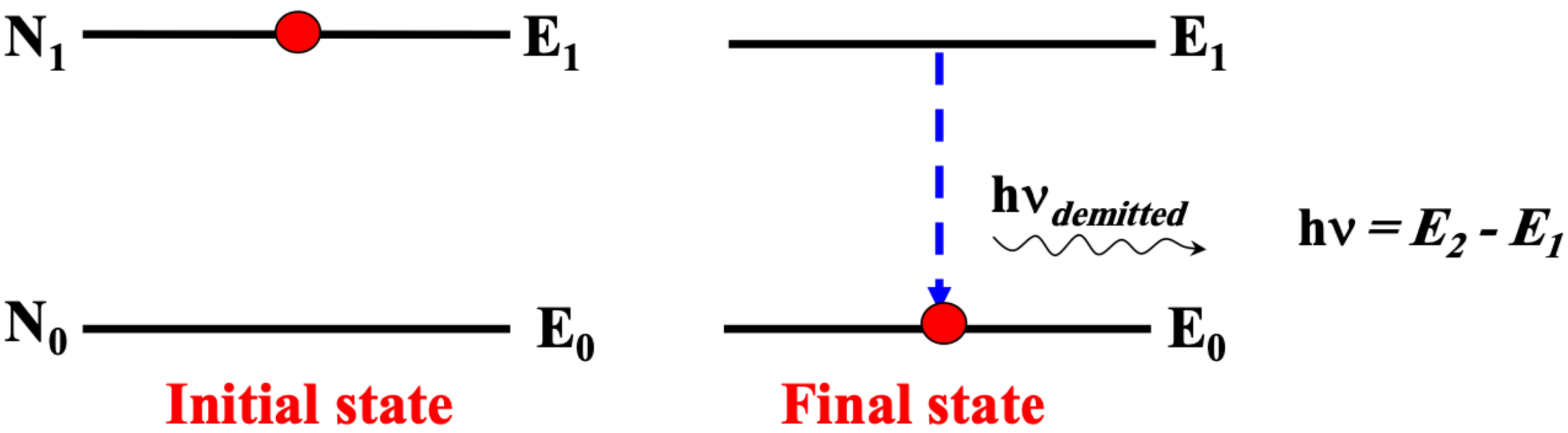
Light-emitting diodes (LEDs)



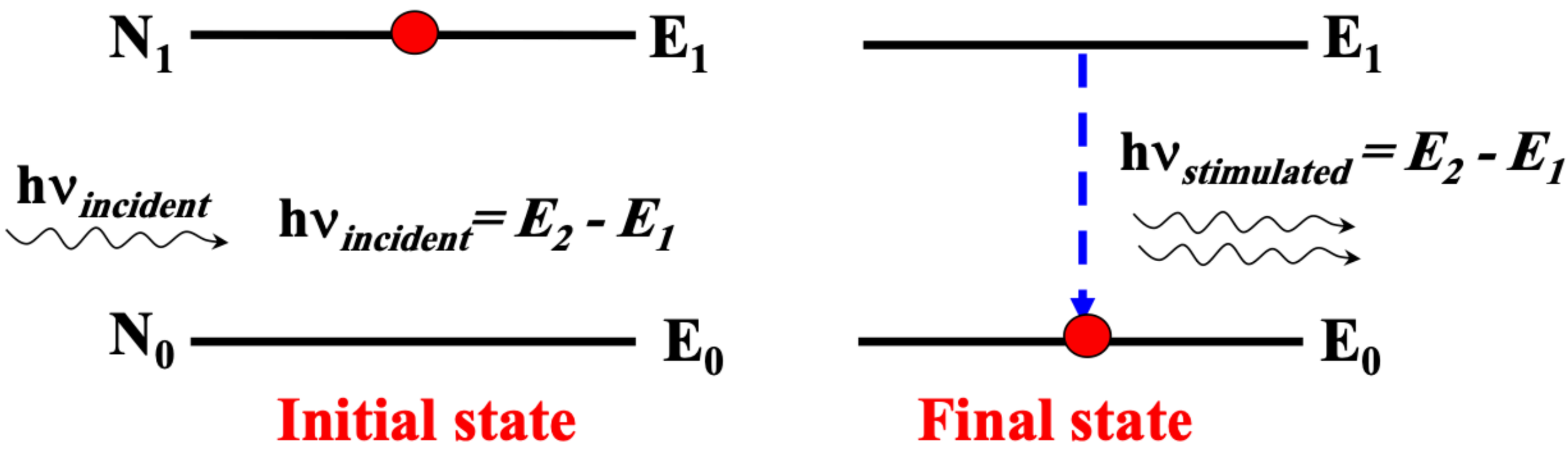


Light guides

Spontaneous emission of radiation

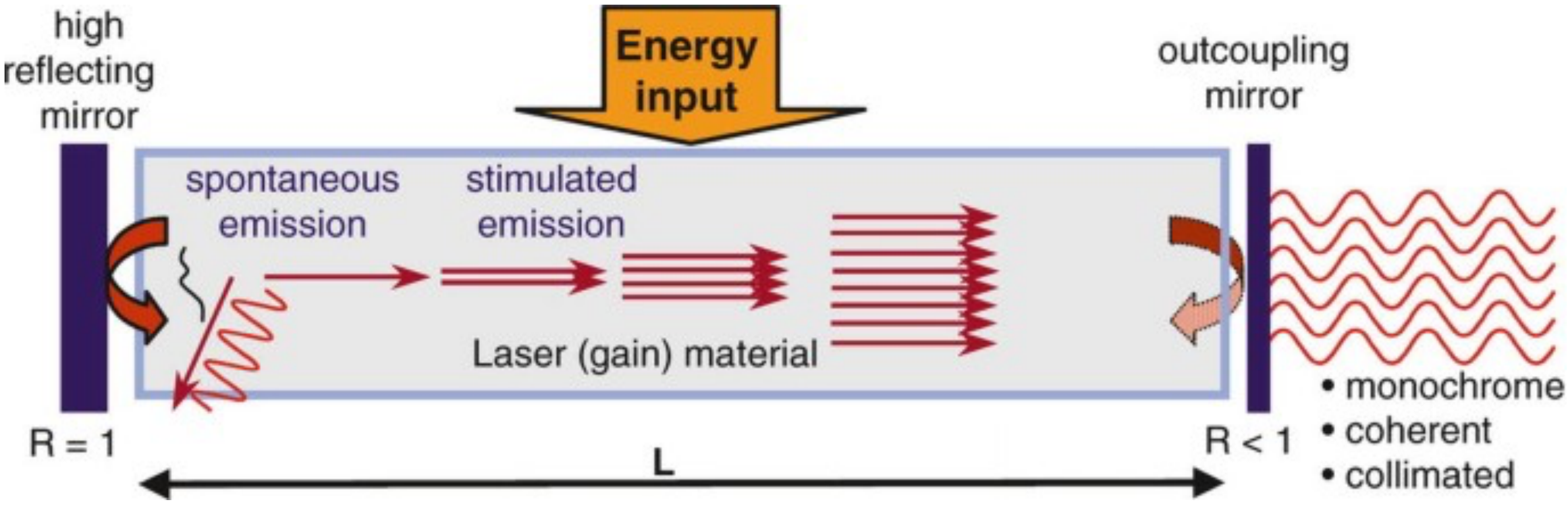


Stimulated Emission of Radiation



LASER

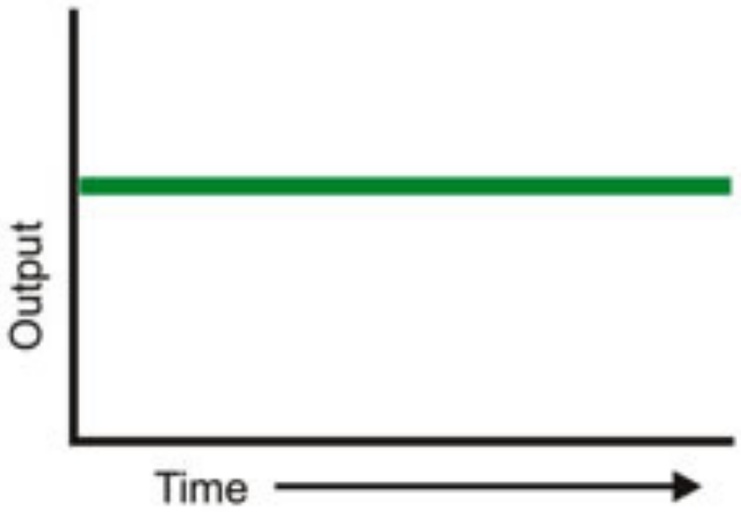
Light Amplification by Stimulated Emission of Radiation



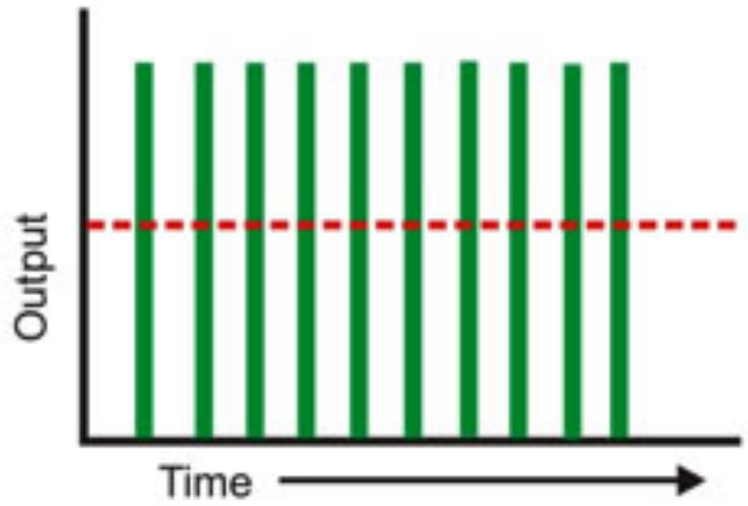
Types of lasers

- Gas Lasers
- Solid-State Lasers
- Fiber Lasers
- Liquid Lasers (Dye Lasers)
- Semiconductor Lasers (Laser Diodes)

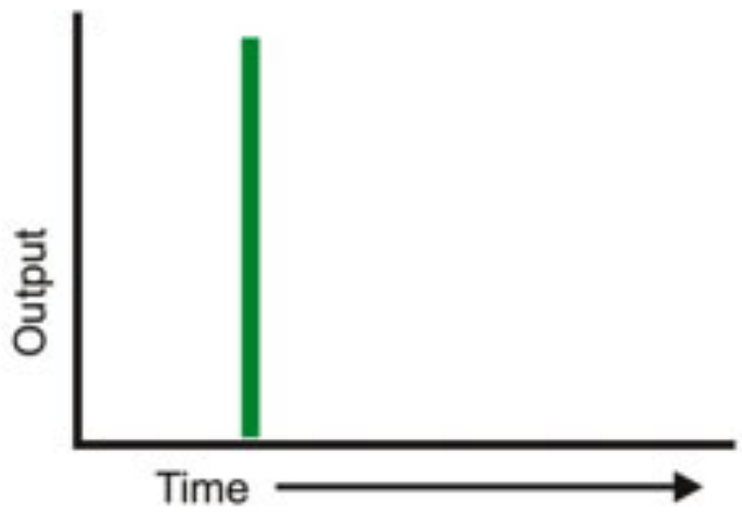
Continuous Wave (CW) vs Pulsed Lasers



Continuous CW Output

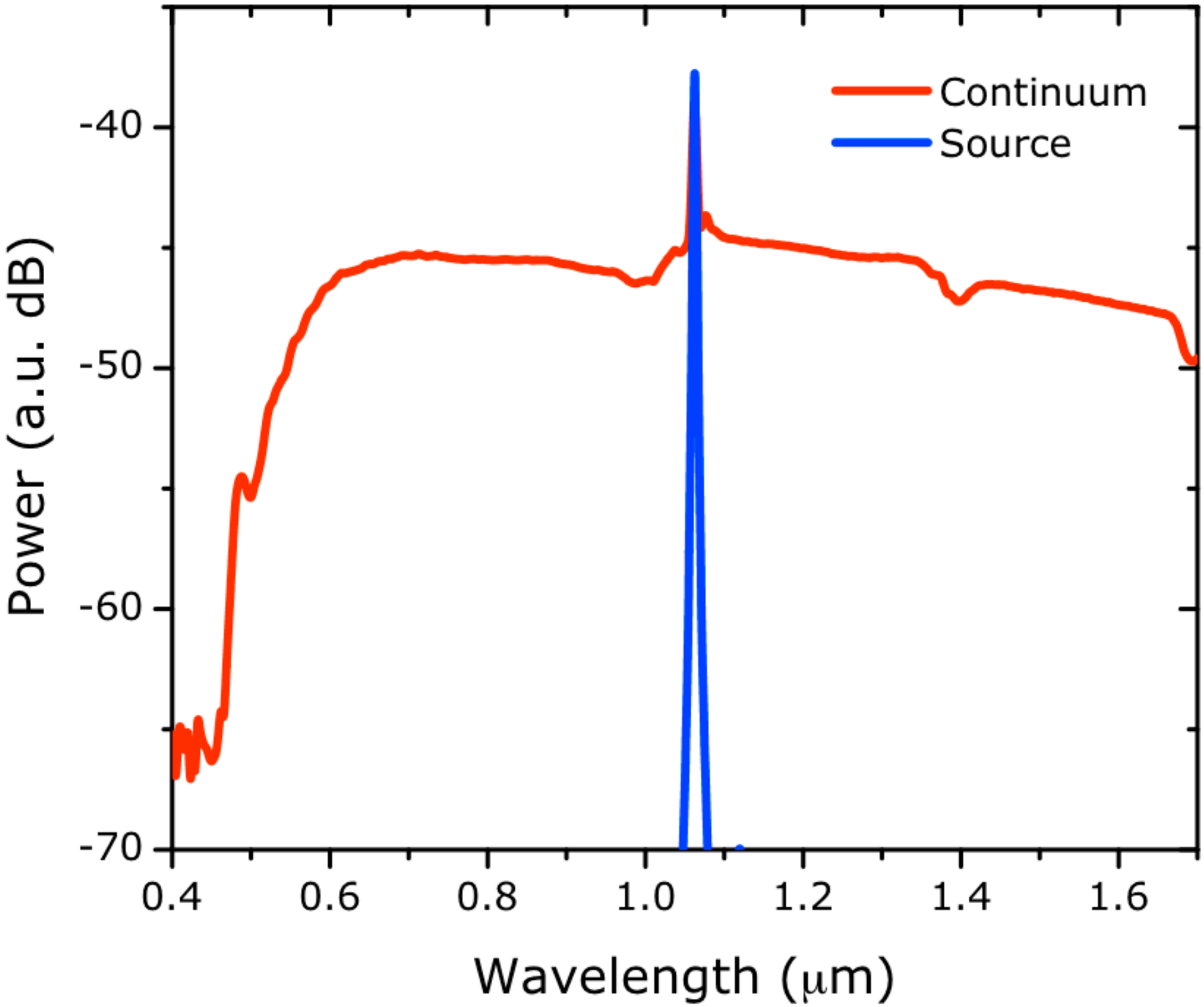


Repetitive Pulse Output



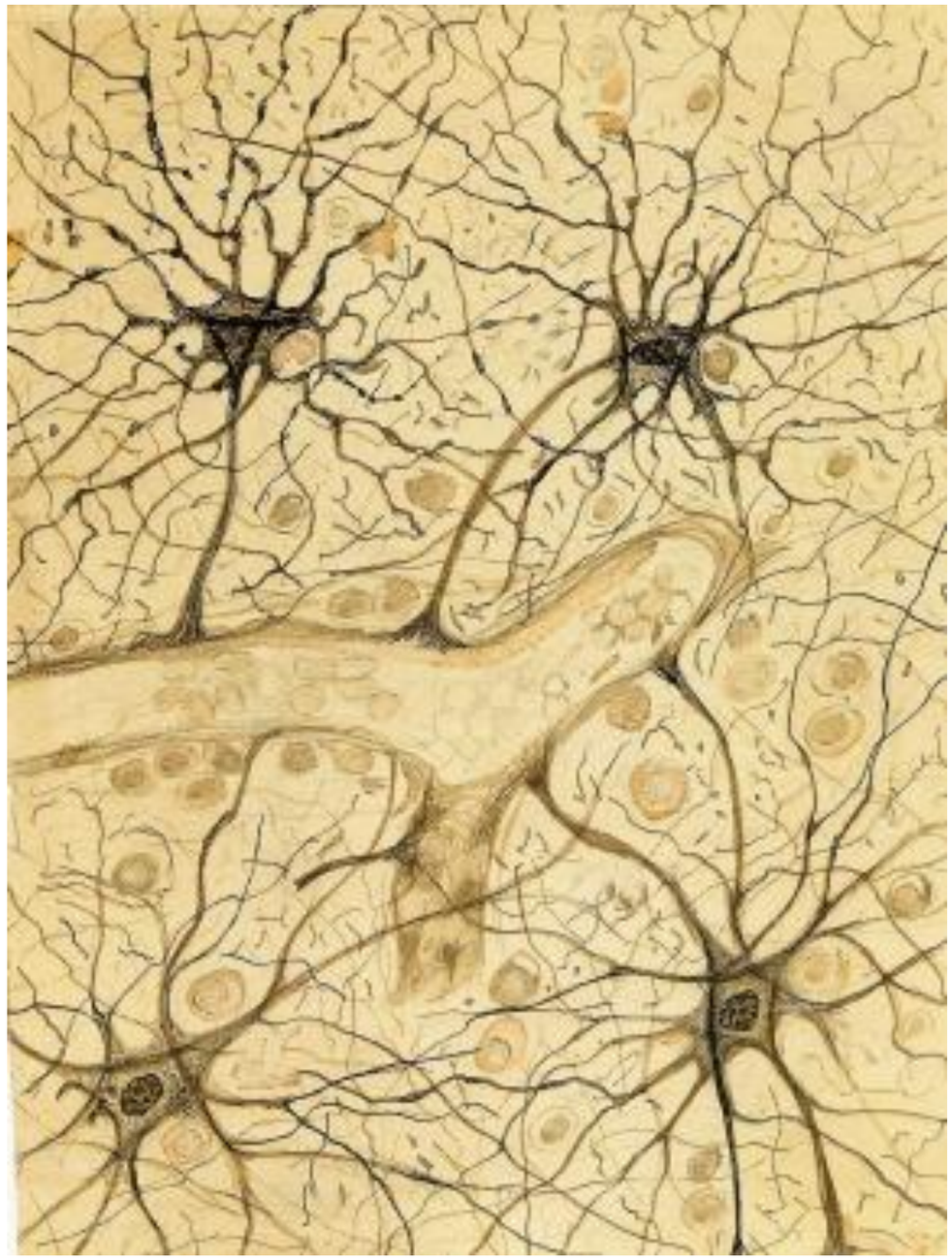
Single Pulse Output

White light super continuum laser



More than meets the eye

Santiago Ramón y Cajal



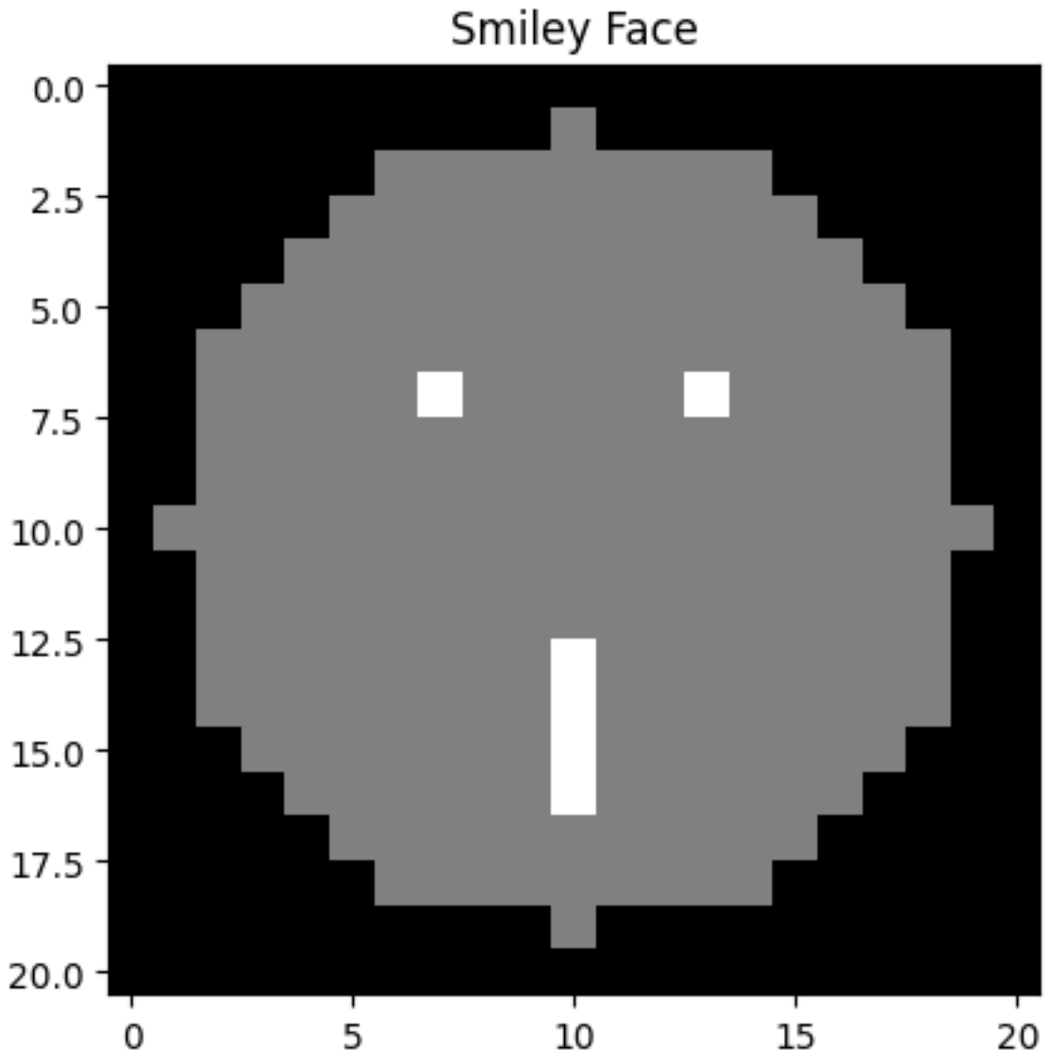
Then there was film cameras

Digital Images

Single Pixel Detector

Multipixel Camera

A digital image is an array of numbers



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0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

A digital image is an array of numbers

0	2	15	0	0	11	10	0	0	0	0	9	9	0	0	0
0	0	0	4	60	157	236	255	255	177	95	61	32	0	0	29
0	10	16	119	238	255	244	245	243	250	249	255	222	103	10	0
0	14	170	255	255	244	254	255	253	245	255	249	253	251	124	1
2	98	255	228	255	251	254	211	141	116	122	215	251	238	255	49
13	217	243	255	155	33	226	52	2	0	10	13	232	255	255	36
16	229	252	254	49	12	0	0	7	7	0	70	237	252	235	62
6	141	245	255	212	25	11	9	3	0	115	236	243	255	137	0
0	87	252	250	248	215	60	0	1	121	252	255	248	144	6	0
0	13	113	255	255	245	255	182	181	248	252	242	208	36	0	19
1	0	5	117	251	255	241	255	247	255	241	162	17	0	7	0
0	0	0	4	58	251	255	246	254	253	255	120	11	0	1	0
0	0	4	97	255	255	255	248	252	255	244	255	182	10	0	4
0	22	206	252	246	251	241	100	24	113	255	245	255	194	9	0
0	111	255	242	255	158	24	0	0	6	39	255	232	230	56	0
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0	107	251	241	255	230	98	55	19	118	217	248	253	255	52	4
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0	0	23	113	215	255	250	248	255	255	248	248	118	14	12	0
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0	0	5	5	0	0	0	0	0	14	1	0	6	6	0	0

0	2	15	0	0	11	10	0	0	0	0	9	9	0	0	0
0	0	0	4	60	157	236	255	255	177	95	61	32	0	0	29
0	10	16	119	238	255	244	245	243	250	249	255	222	103	10	0
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2	98	255	228	255	251	254	211	141	116	122	215	251	238	255	49
13	217	243	255	155	33	226	52	2	0	10	13	232	255	255	36
16	229	252	254	49	12	0	0	7	7	0	70	237	252	235	62
6	141	245	255	212	25	11	9	3	0	115	236	243	255	137	0
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0	0	0	4	58	251	255	246	254	253	255	120	11	0	1	0
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0	22	206	252	246	251	241	100	24	113	255	245	255	194	9	0
0	111	255	242	255	158	24	0	0	6	39	255	232	230	56	0
0	218	251	250	137	7	11	0	0	0	2	62	255	250	125	3
0	173	255	255	101	9	20	0	13	3	13	182	251	245	61	0
0	107	251	241	255	230	98	55	19	118	217	248	253	255	52	4
0	18	146	250	255	247	255	255	255	249	255	240	255	129	0	5
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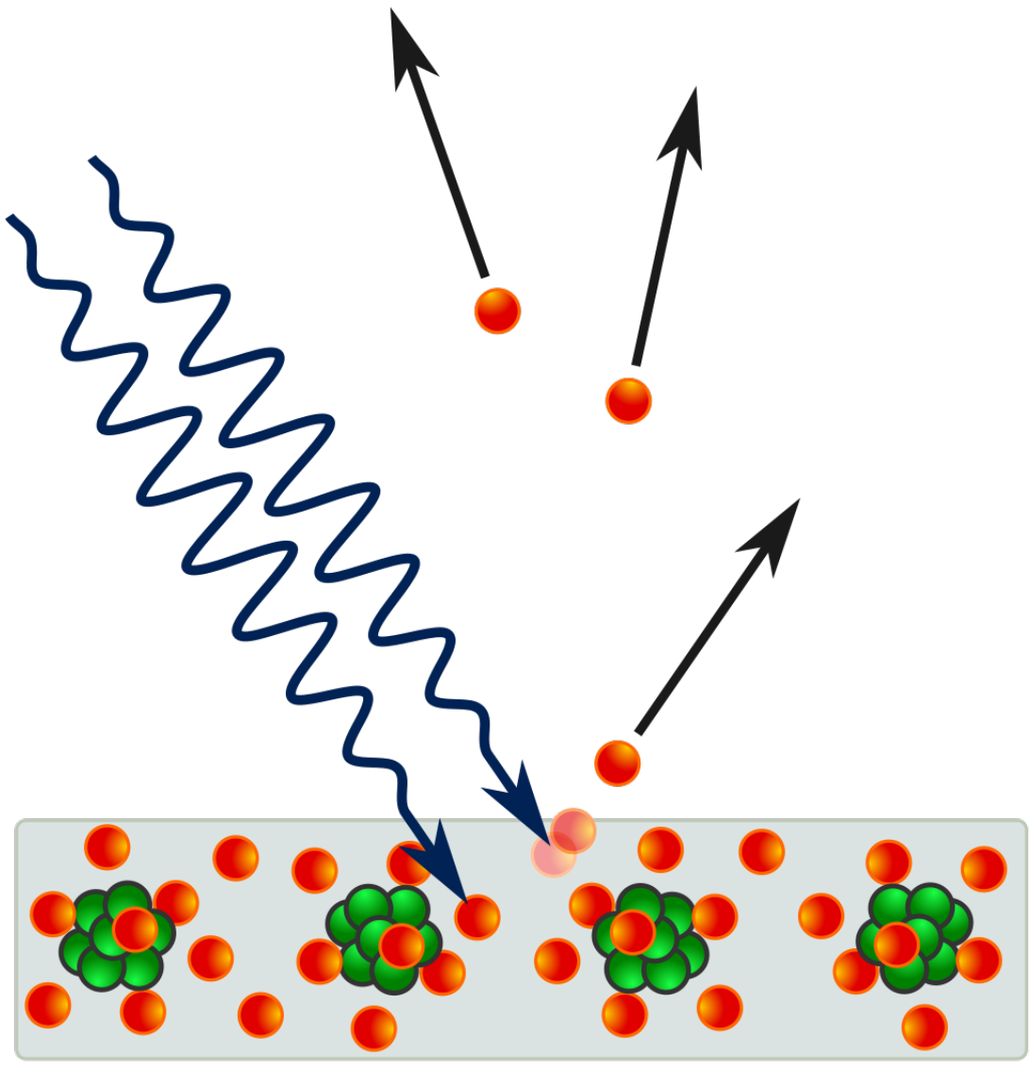
0	0	6	1	0	52	153	233	255	252	147	37	0	0	4	1
0	0	5	5	0	0	0	0	0	14	1	0	6	6	0	0

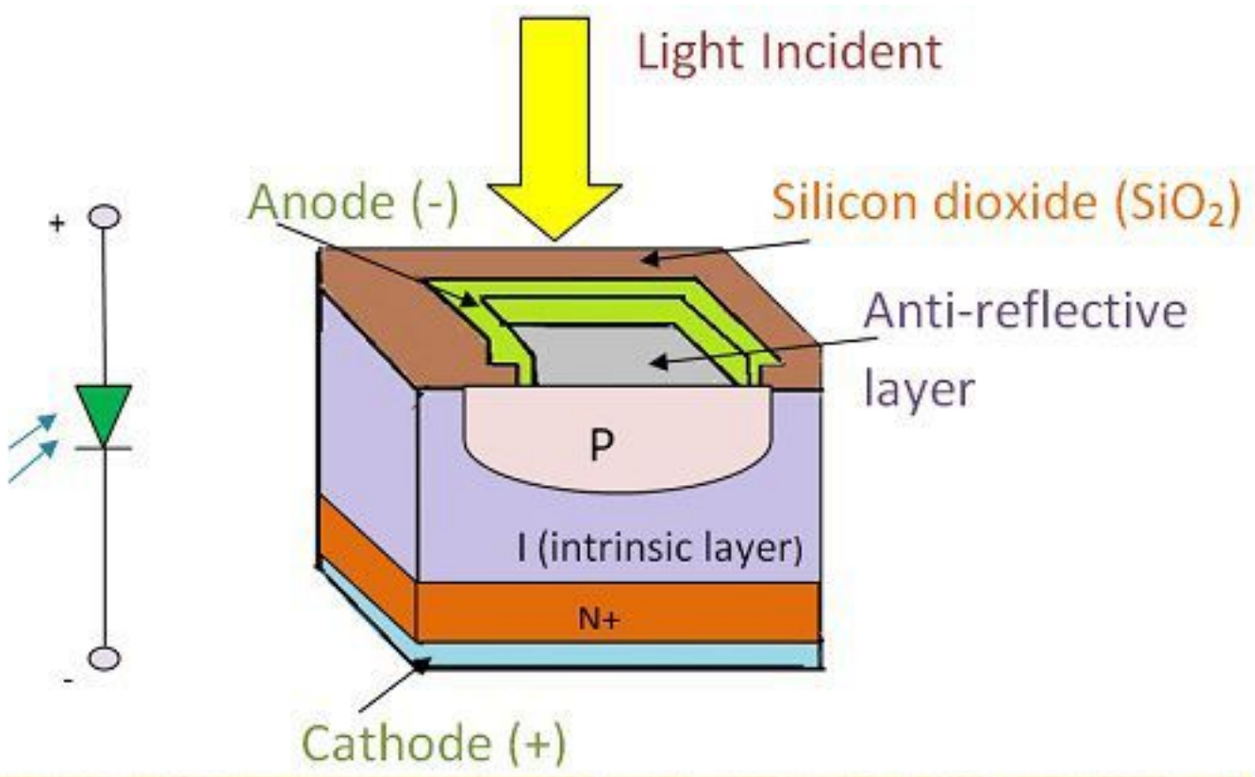
Converting Photons to Numbers

Step 1: Photons to electrons

Photoelectric effect

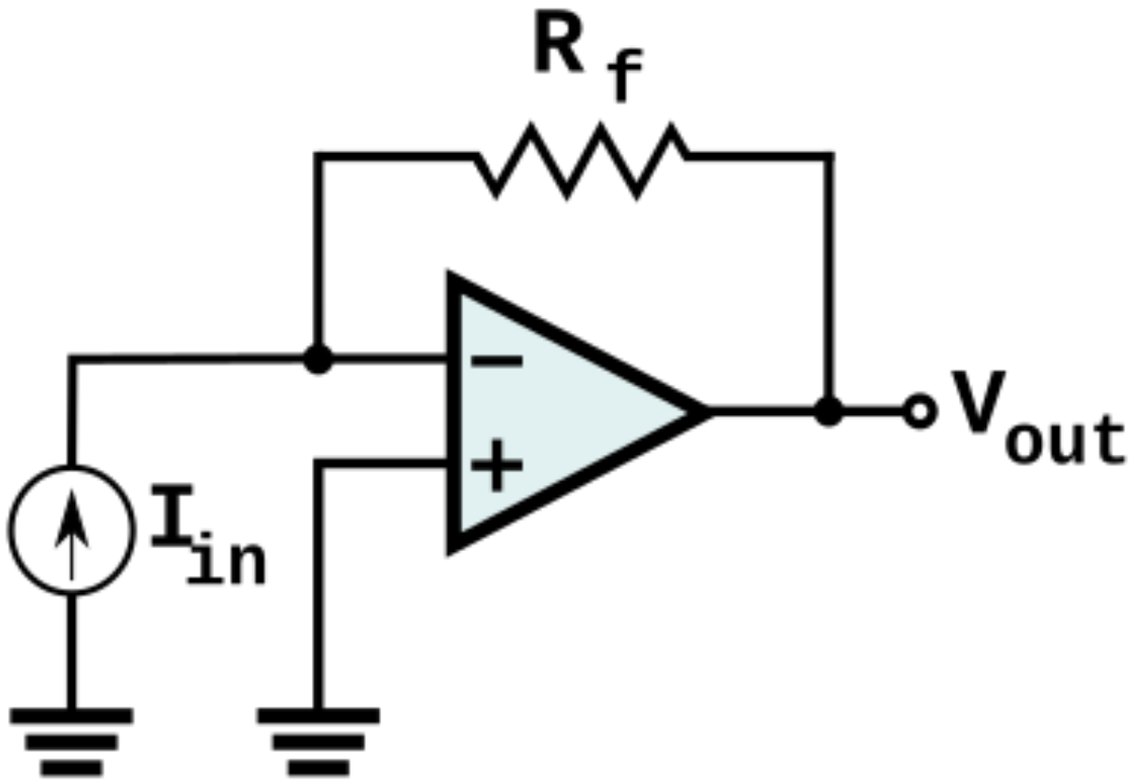


Photodiode

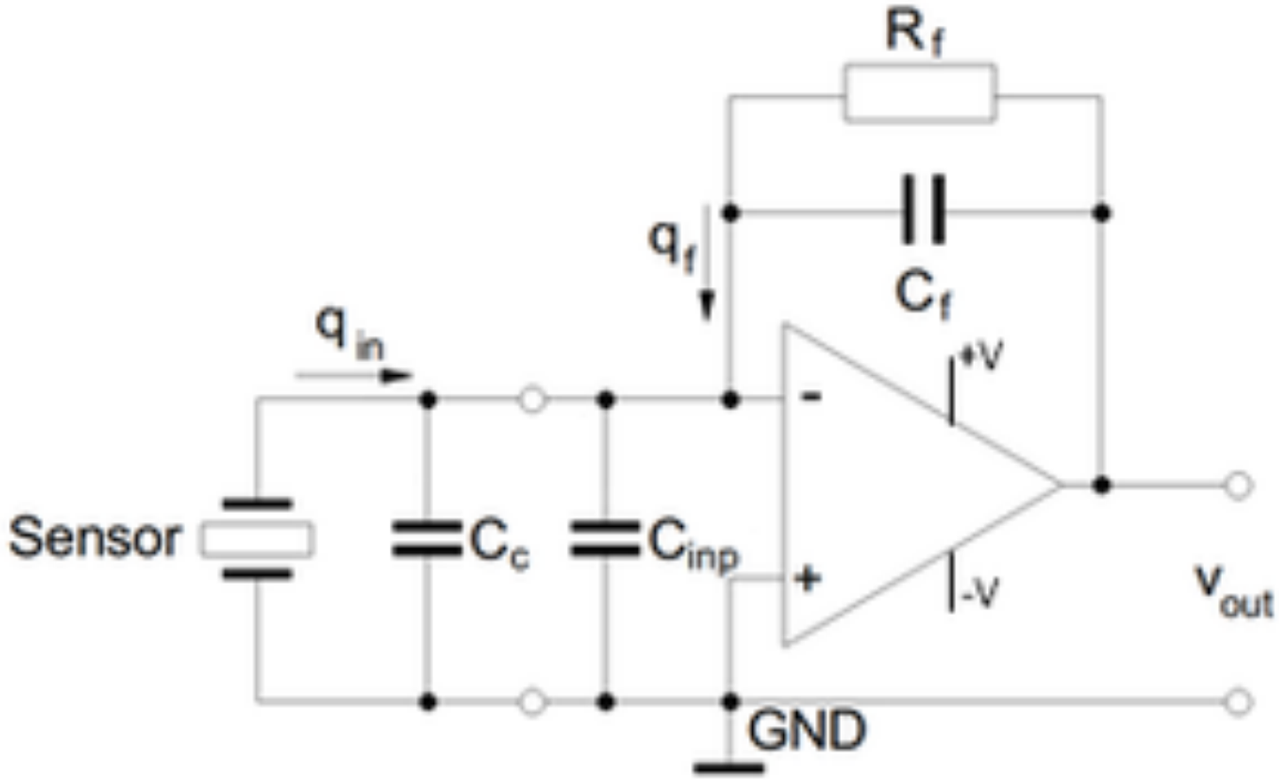


Converting Photons to Numbers

Step 2: Current/Charge to Voltage

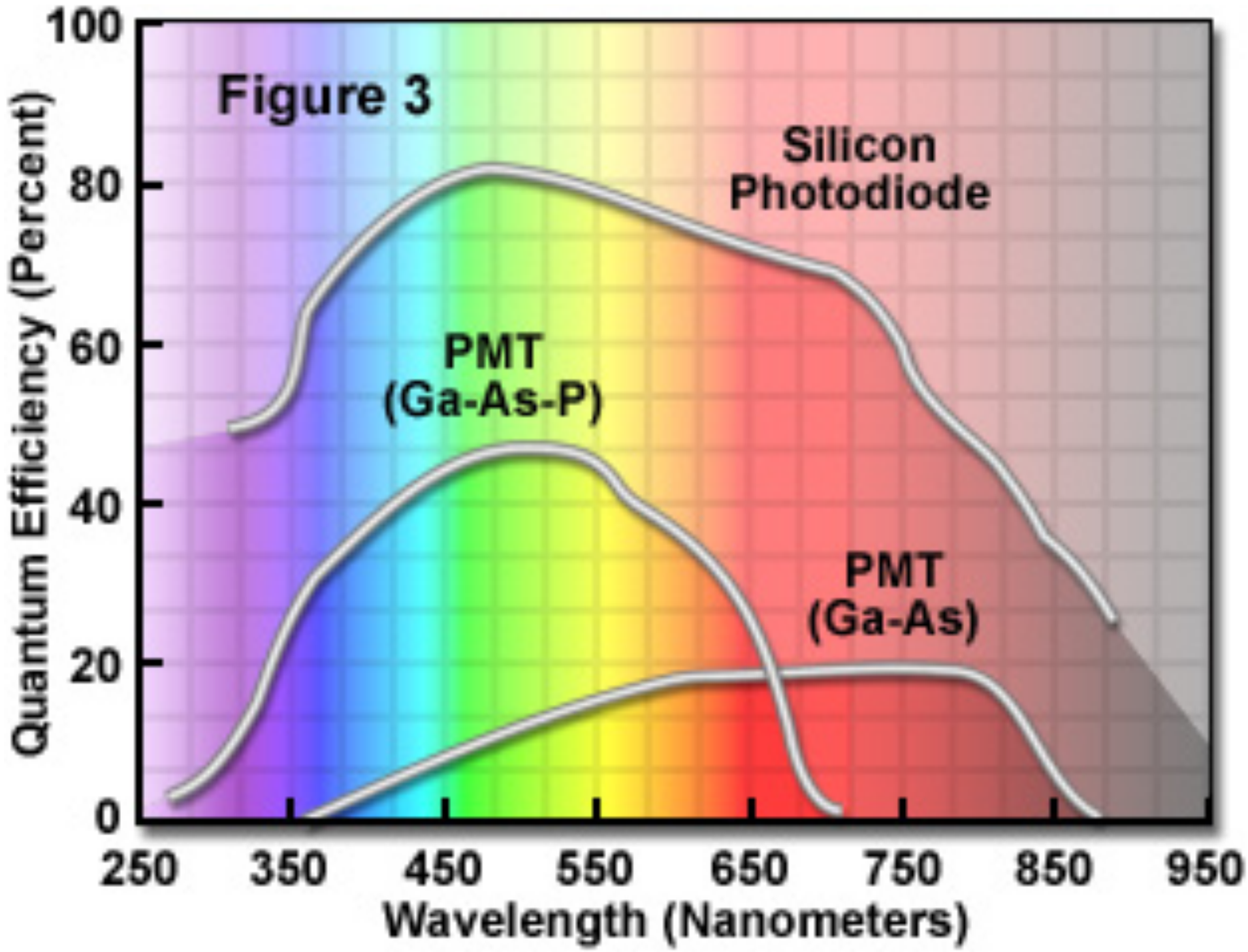


Transimpedance Amplifier



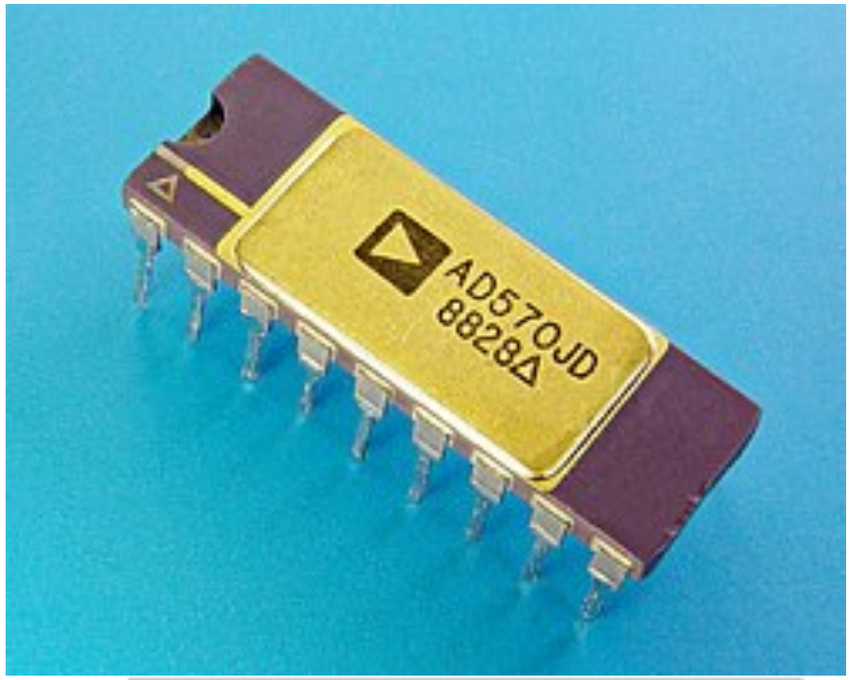
Charge Amplifier

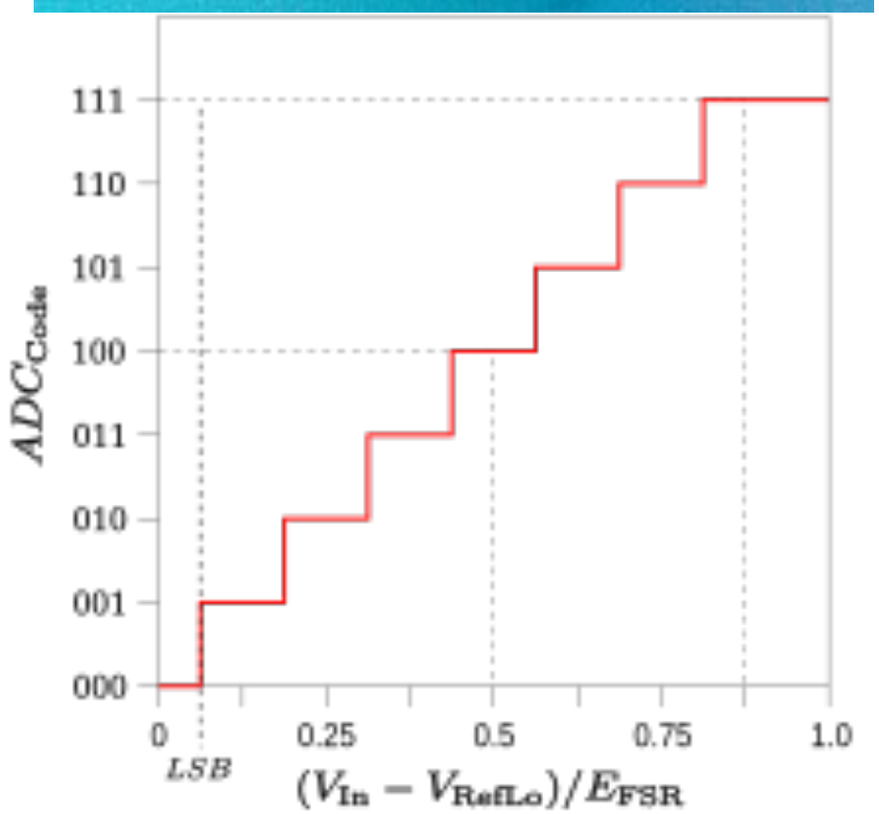
Quantum efficiency



Converting Photons to Numbers

Step 3: Analog to Digital Converter (ADC)





$$\text{Number of Levels} = 2^{\text{Number of Bits}}$$

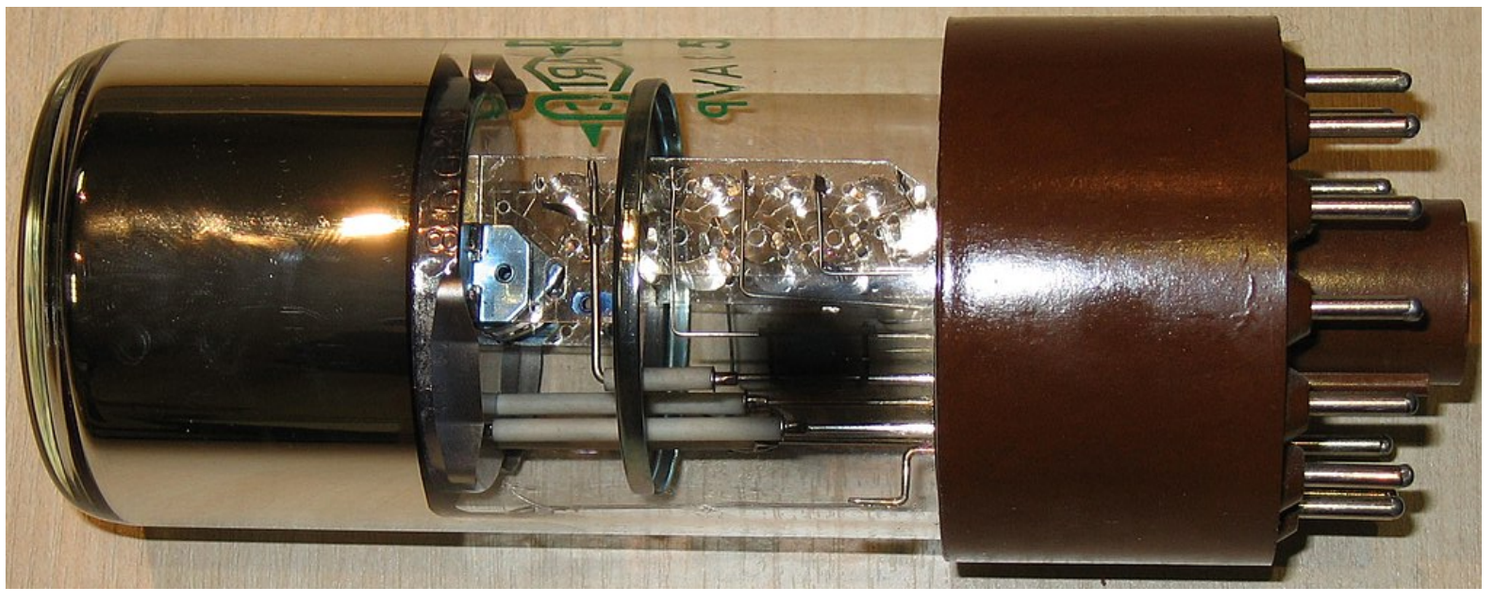
Bits	Number of Levels
8	$2^8 = 64$
12	$2^{12} = 4096$
14	$2^{14} = 16{,}384$
16	$2^{16} = 65{,}536$

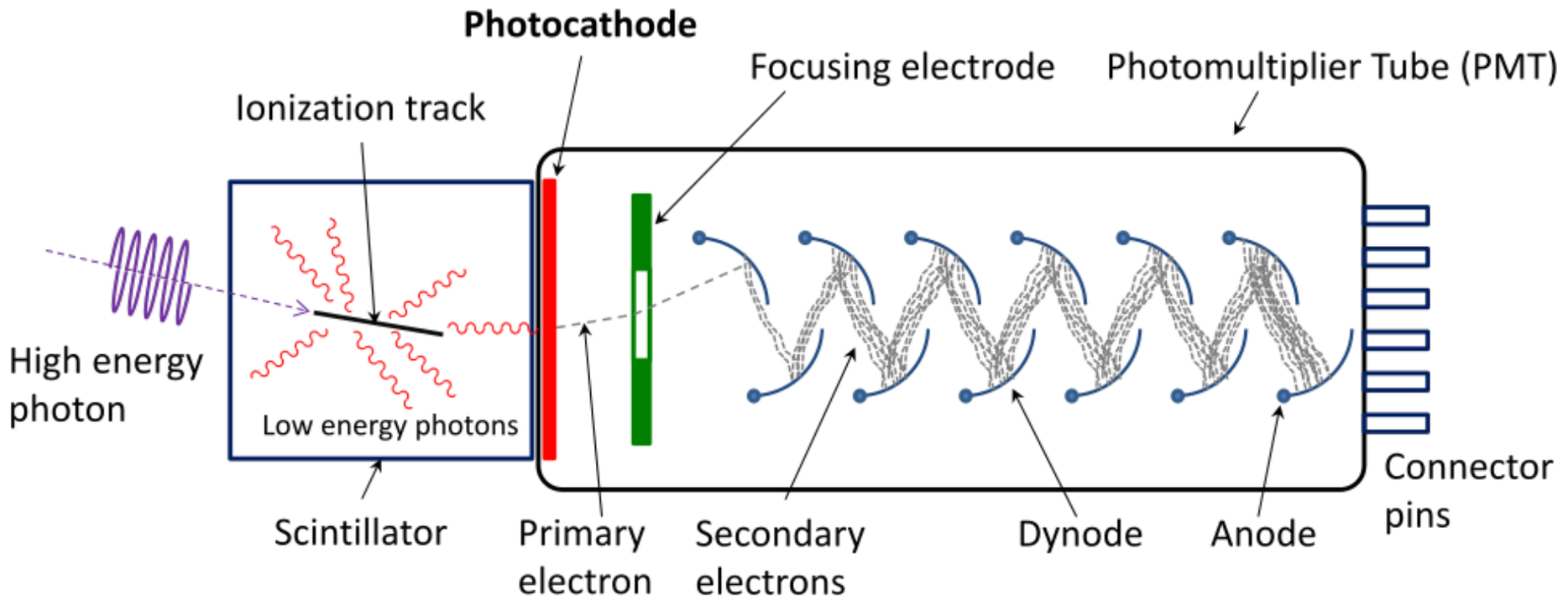
Single Pixel Detectors

Photomultiplier Tube

High gain, linear fast response, ~noisy

Quantum Efficiency~25%





Single Pixel Detectors

Avalanche Photodiode

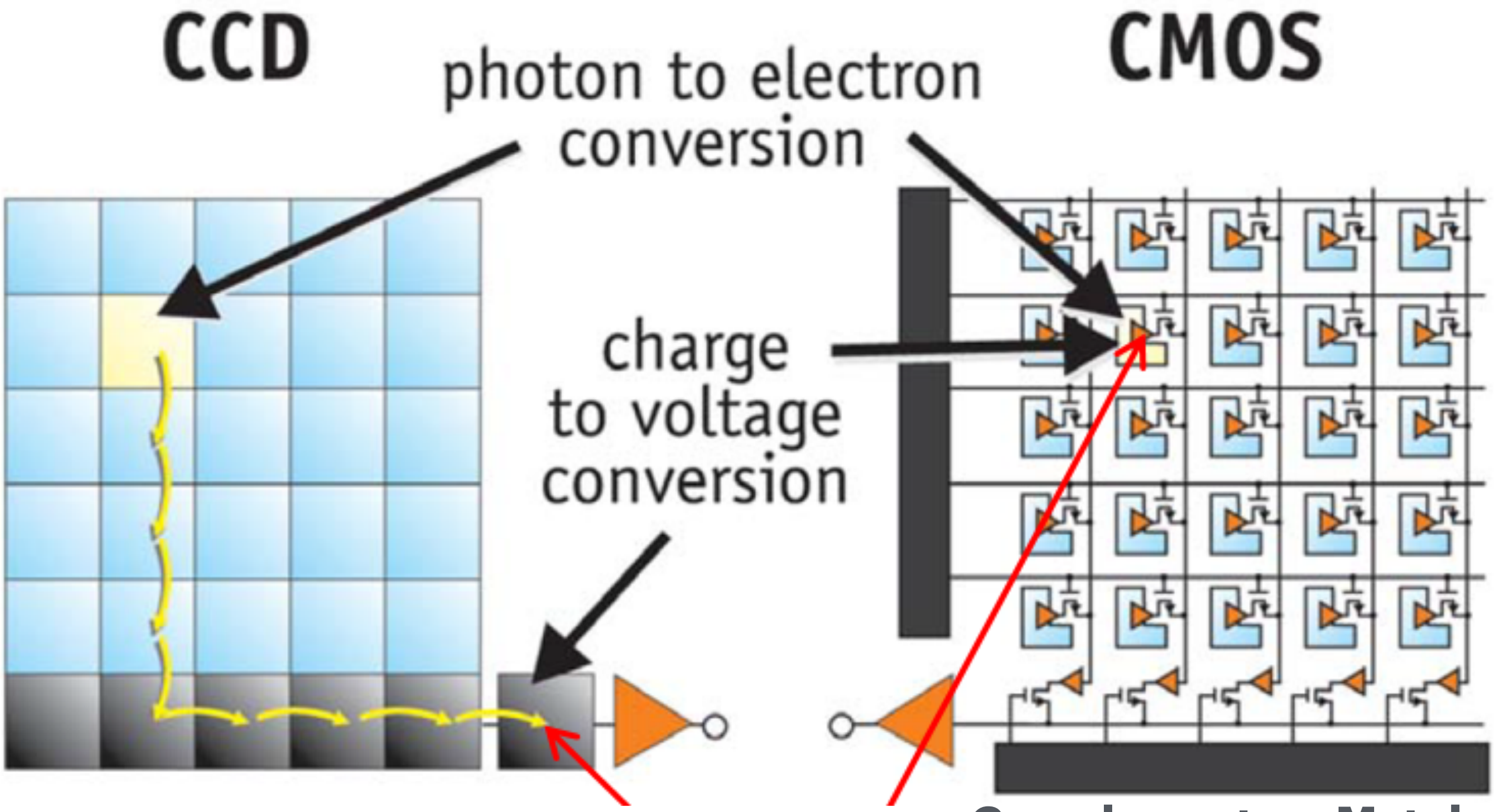
High reverse voltage with photodiode.

Large electric field, free electrons accelerated and generate secondary electrons, “Impact Ionization”

Produces an “avalanche” of change -> Amplification

Quantum Efficiency as high as 90%

Pixelated detectors



Charged Coupled Device

One readout for all pixels

slow, precise, uniform gain

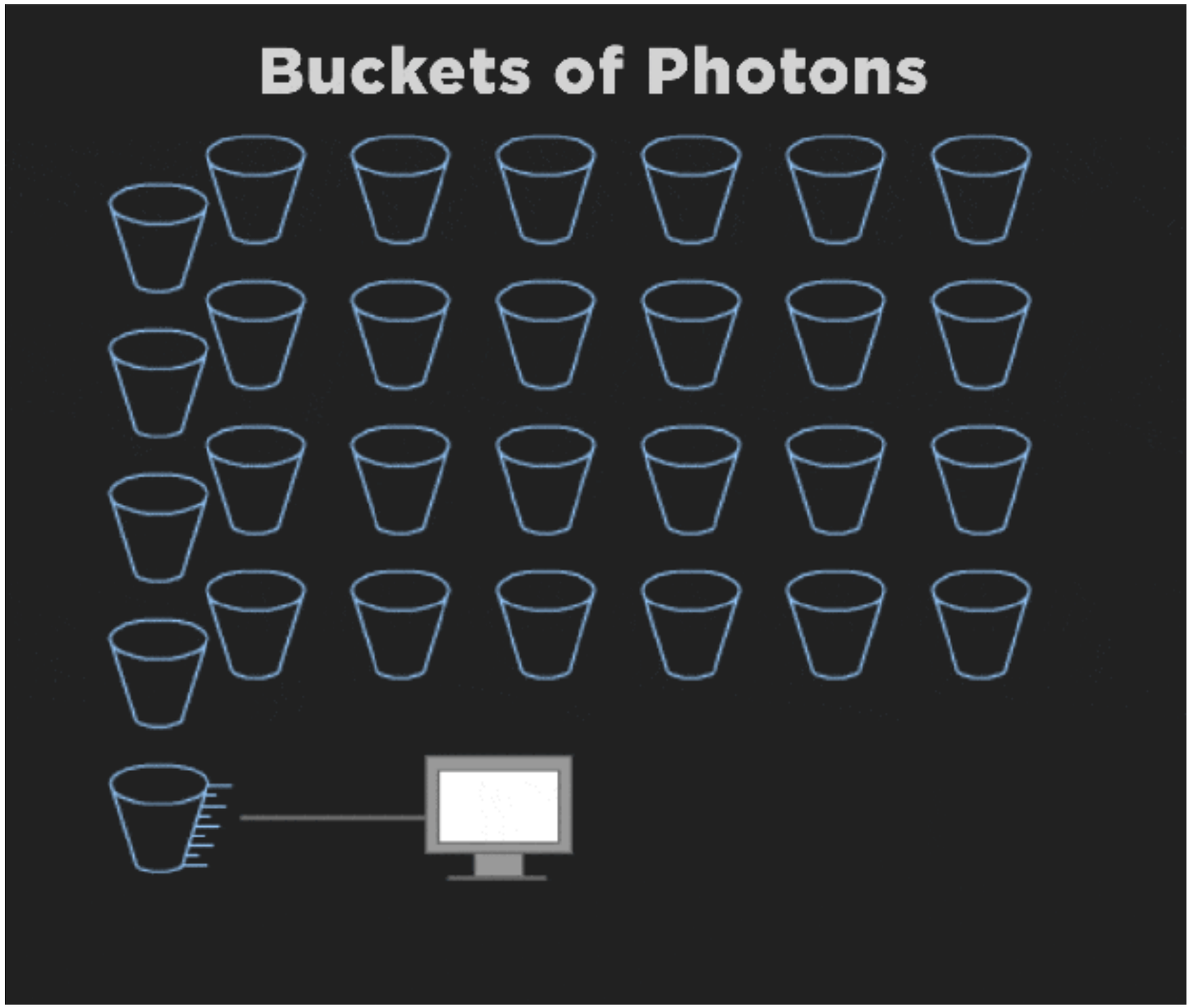
Complementary Metal Oxide Semiconductor

Indep readout on each pixel

fast but noisy

CCD Readout schemes

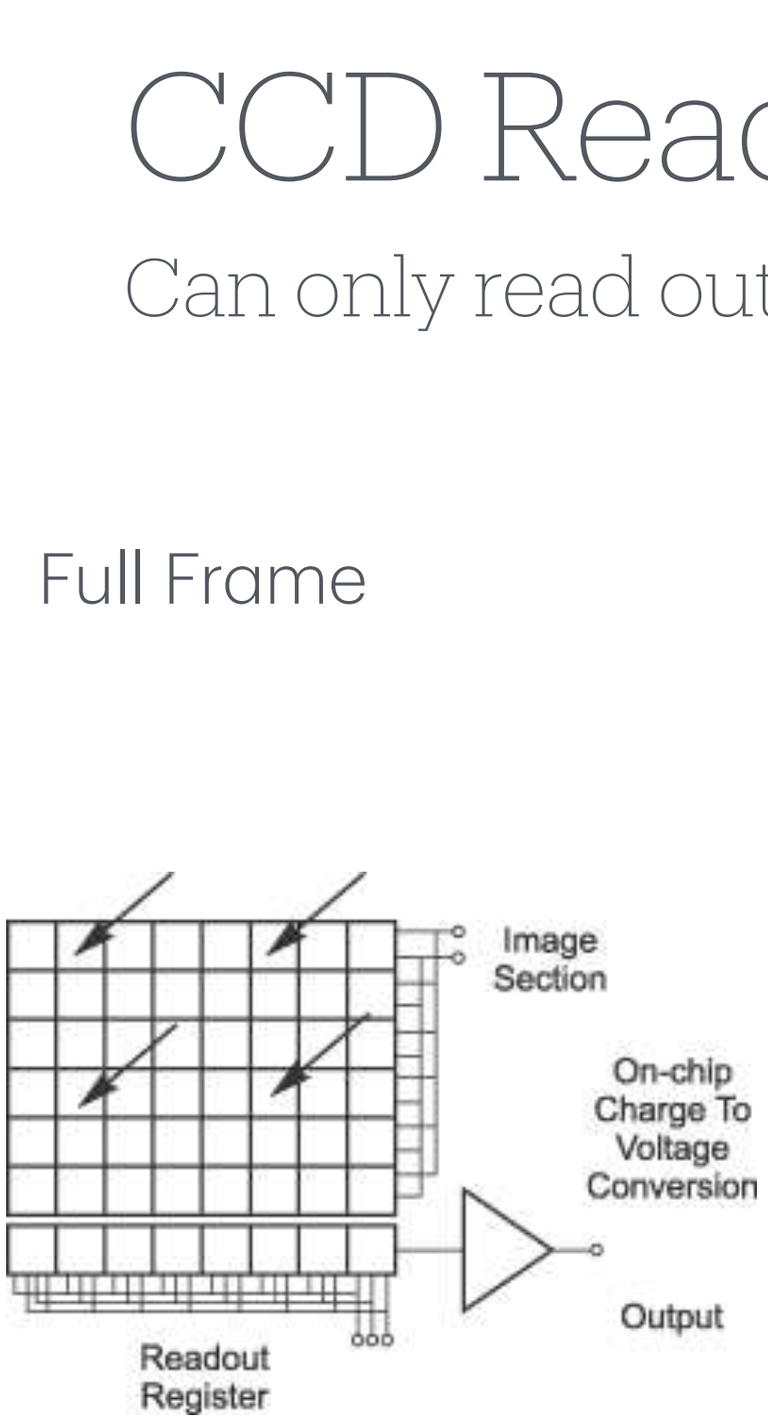
Can only read out one pixel at a time...



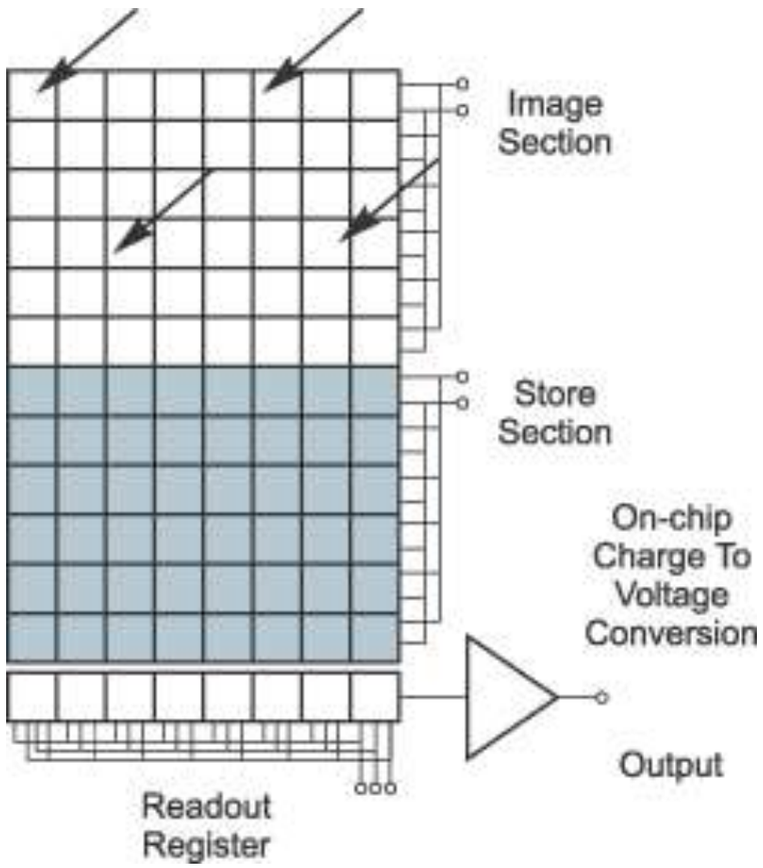
CCD Readout schemes

Can only read out one pixel at a time...

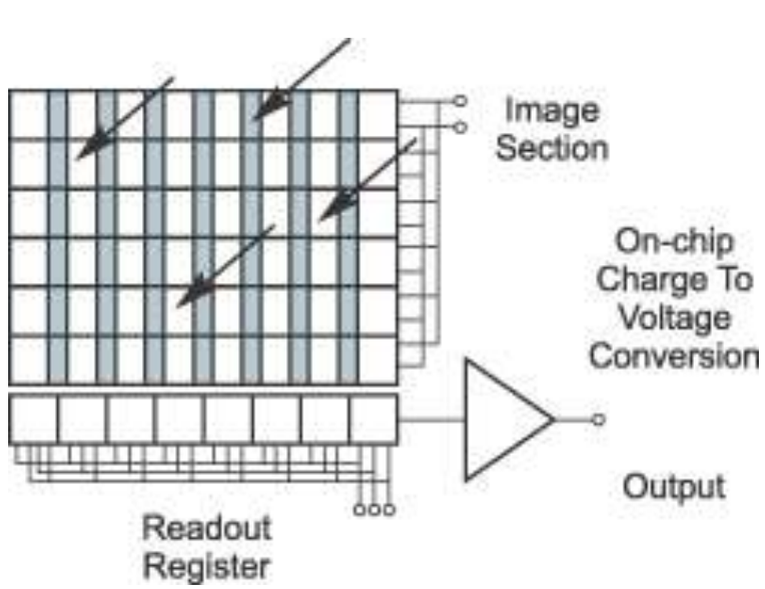
Full Frame



Frame Transfer

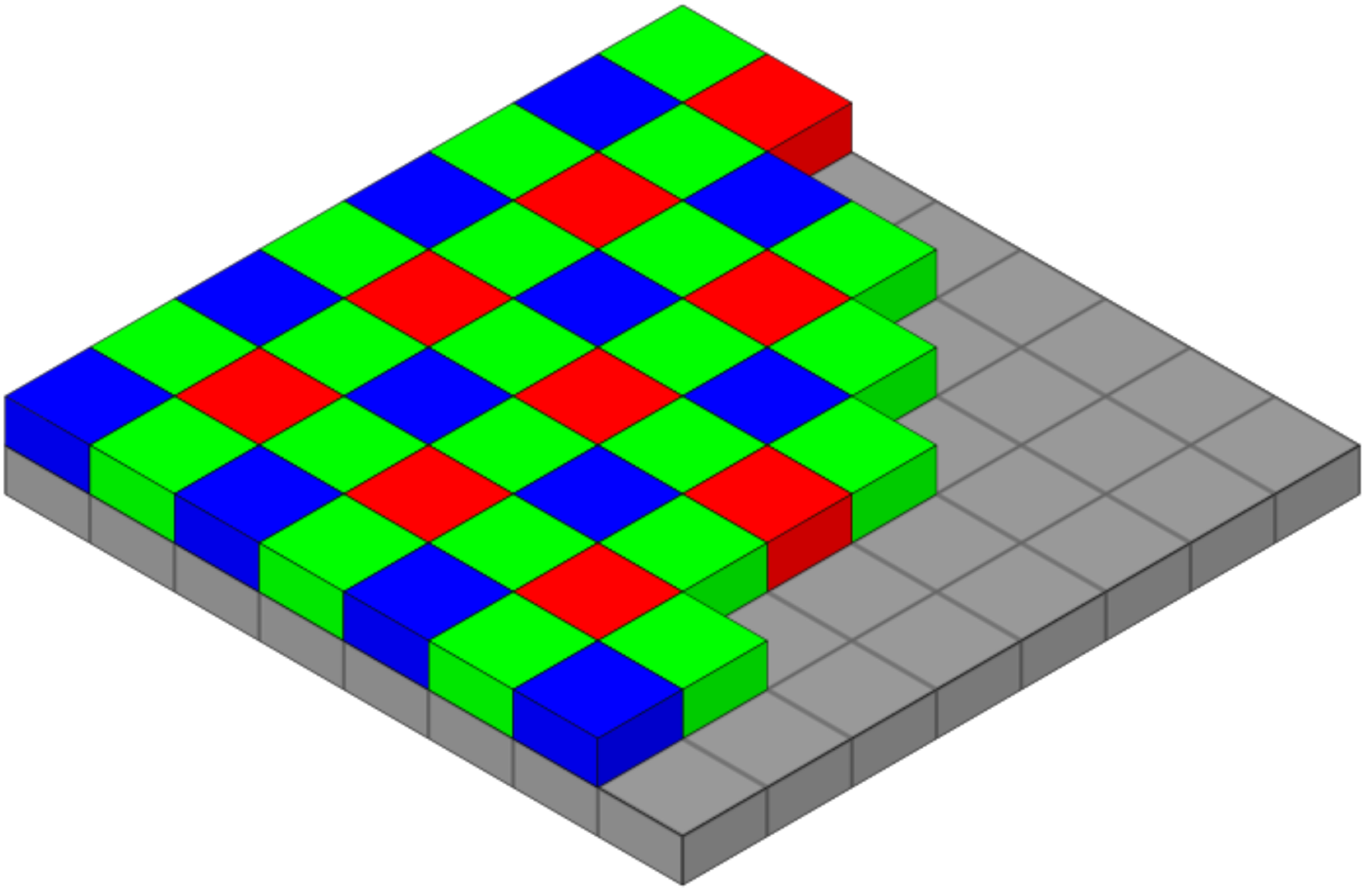


Interline Transfer

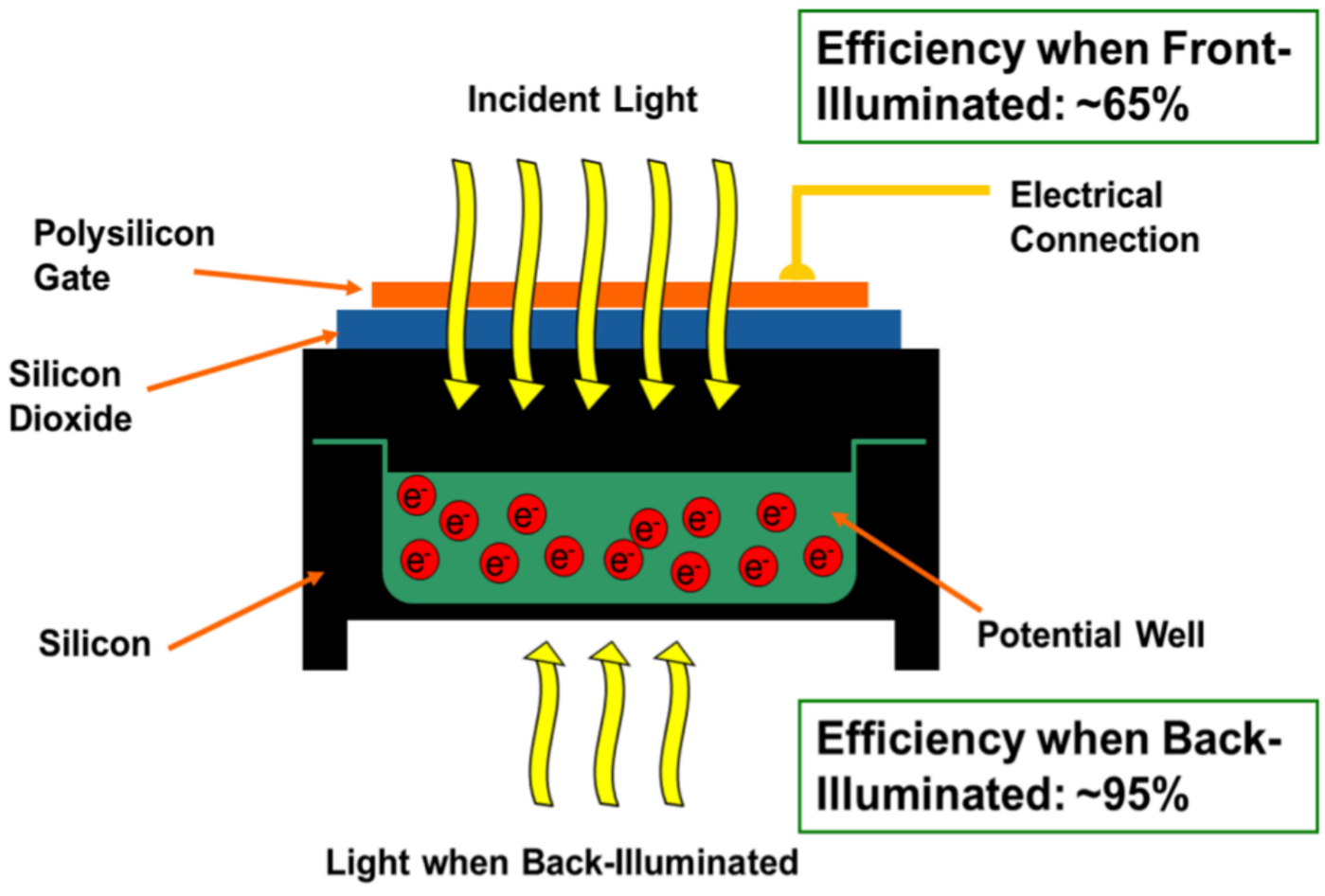


What about color cameras?

You lose at least 1/3 of your light!



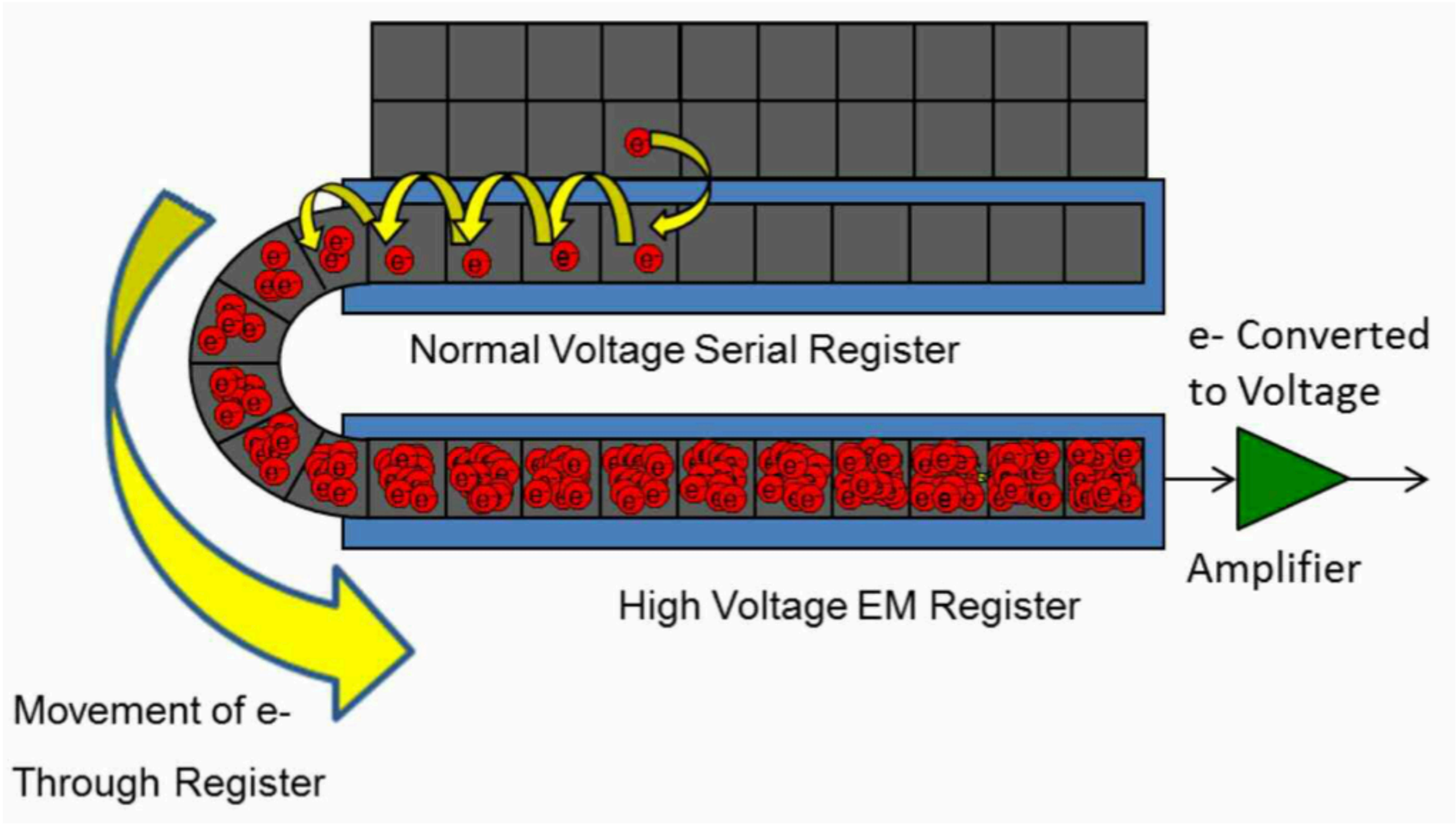
Front vs Backside Illumination



- **Front-side illumination**: light must pass through several layers before reaching the silicon -> light loss

- **Back-illuminated sensor** orientates the wiring behind the photocathode which improves the chance of an input photon being captured from about 60% to over 90%

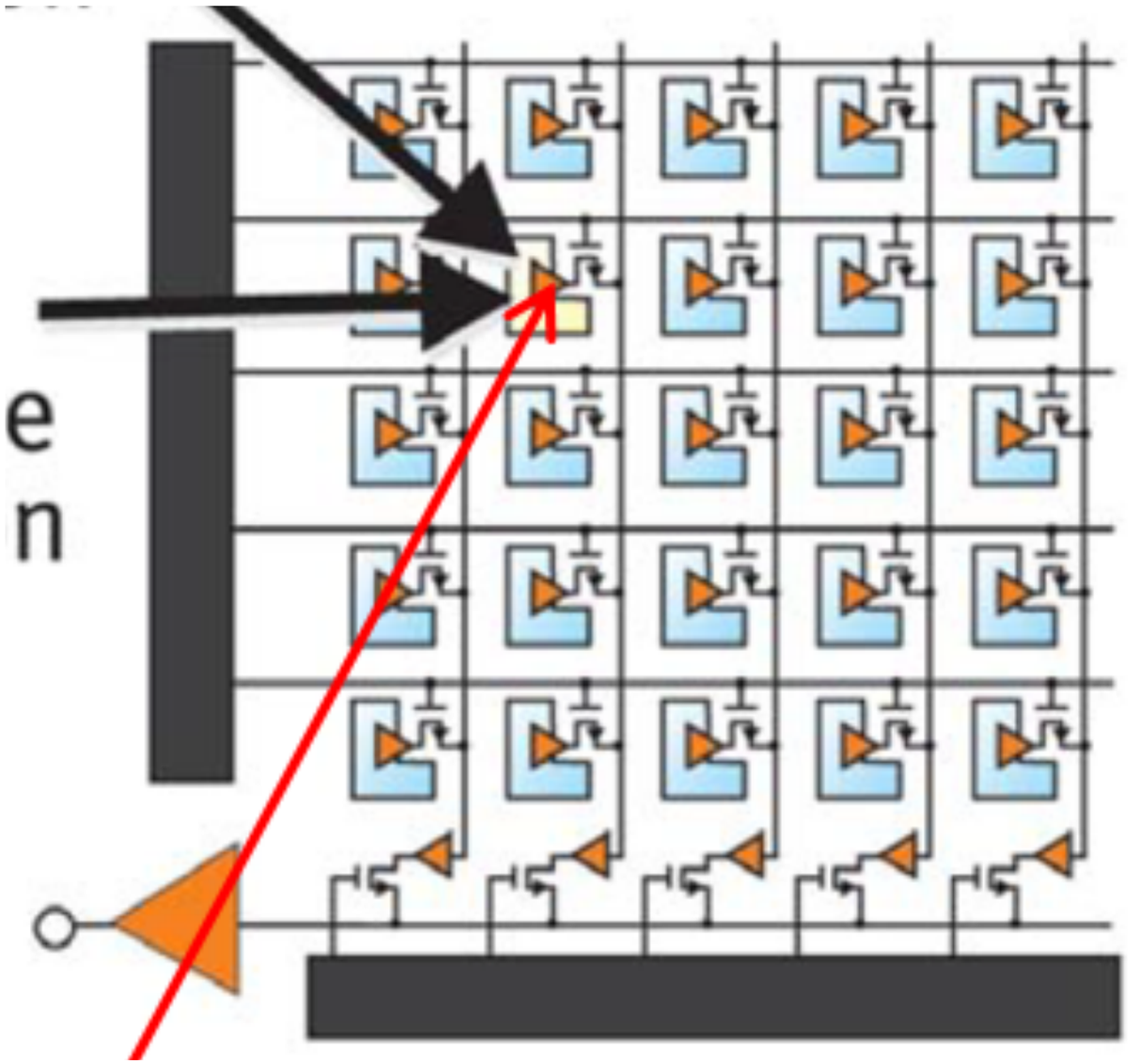
Electron Multiplication (EMCCD)



EM Gain increases signal
lowers effective readout noise
Issues: Dark noise (need to cool the camera), Gain stability

Scientific sCMOS

Next generation of CMOS

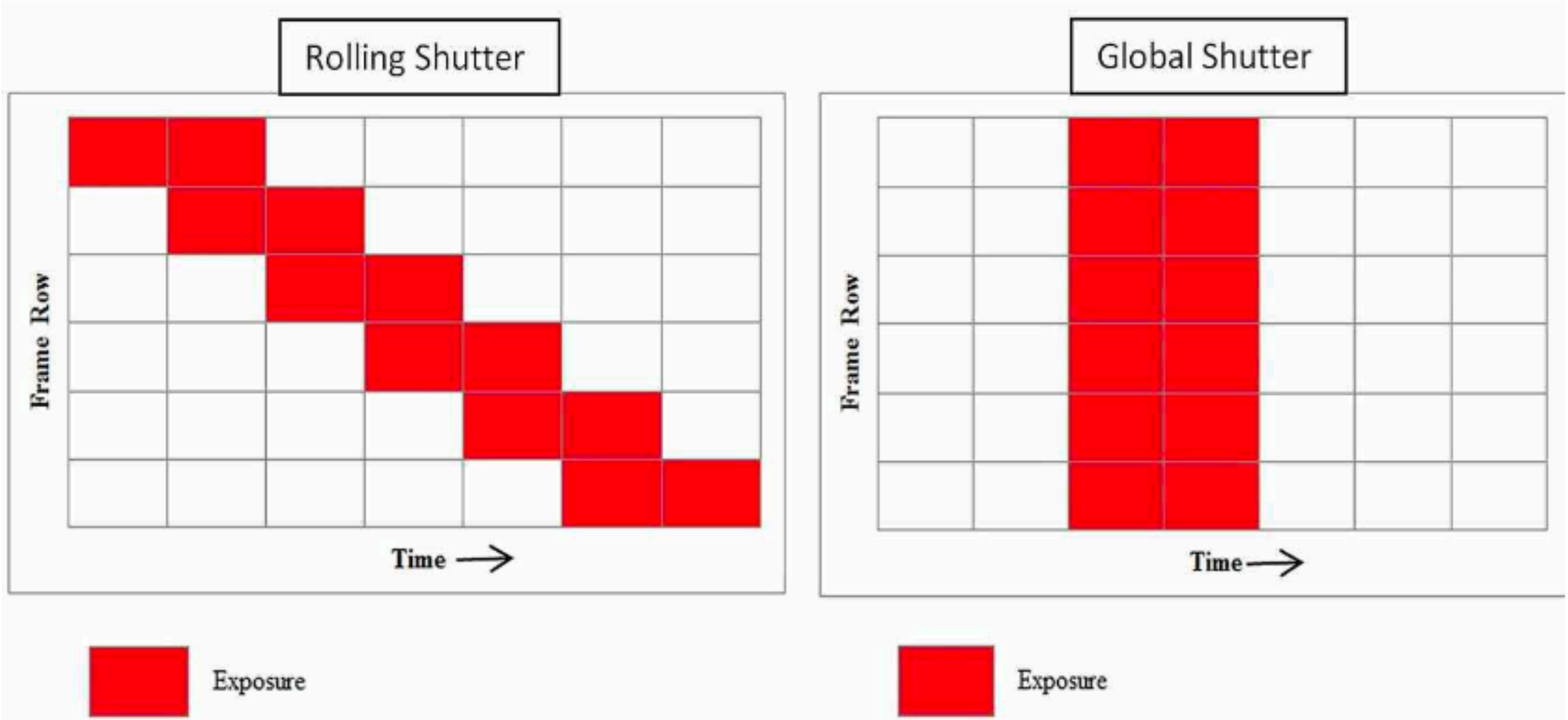


~1 electron read noise, very low

large chips, 2,000x2,000 px

fast 100fps

Readout patterns



Rolling Shutter Artifacts

Signal to Noise (SNR)

Photon/Shot Noise - Stochastic fluctuations from finite number of photons, $\sim \sqrt{N_{photon}}$

Dark Current (DC) - Noise even without photons, ~Temperature -> Cooling

Read Noise (σ_R) - Noise in ADC/amplifier

$$SNR = \frac{\text{Signal} * QE}{\sqrt{\text{Signal} * QE + \text{DC} * t_{\text{exposure}} + \sigma_R^2}}$$

Manual stages

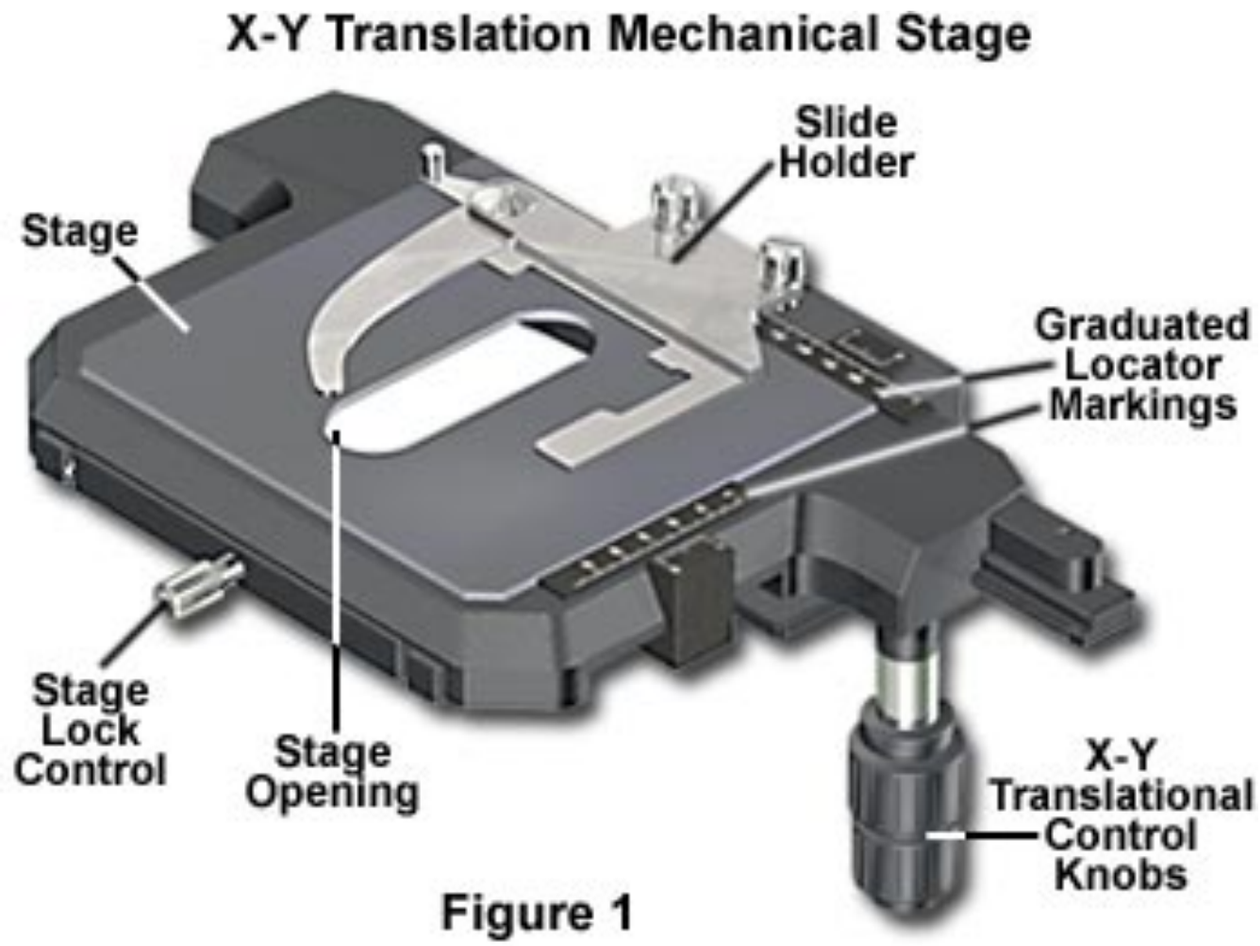
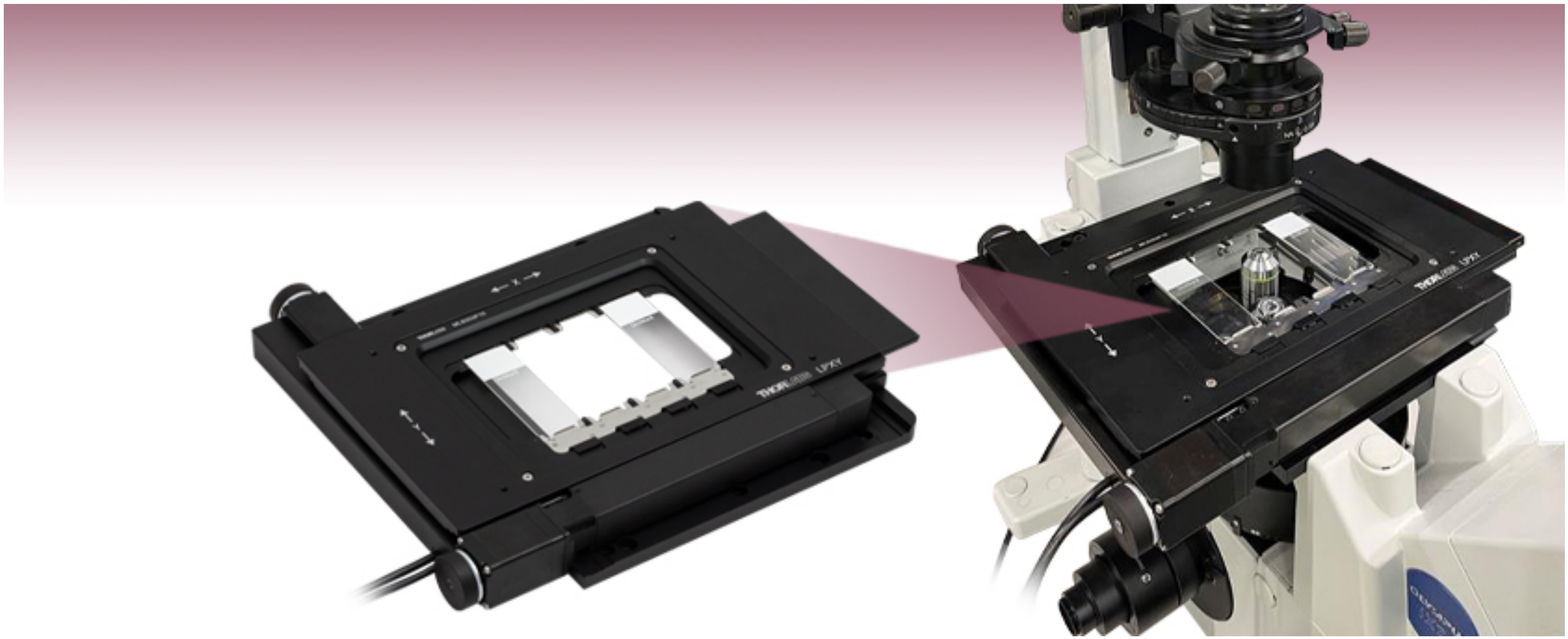


Figure 1

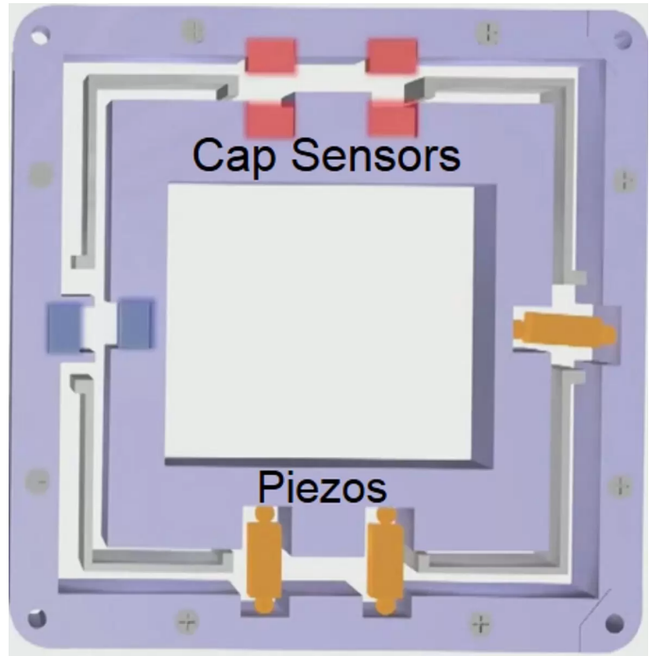
Motorized stages

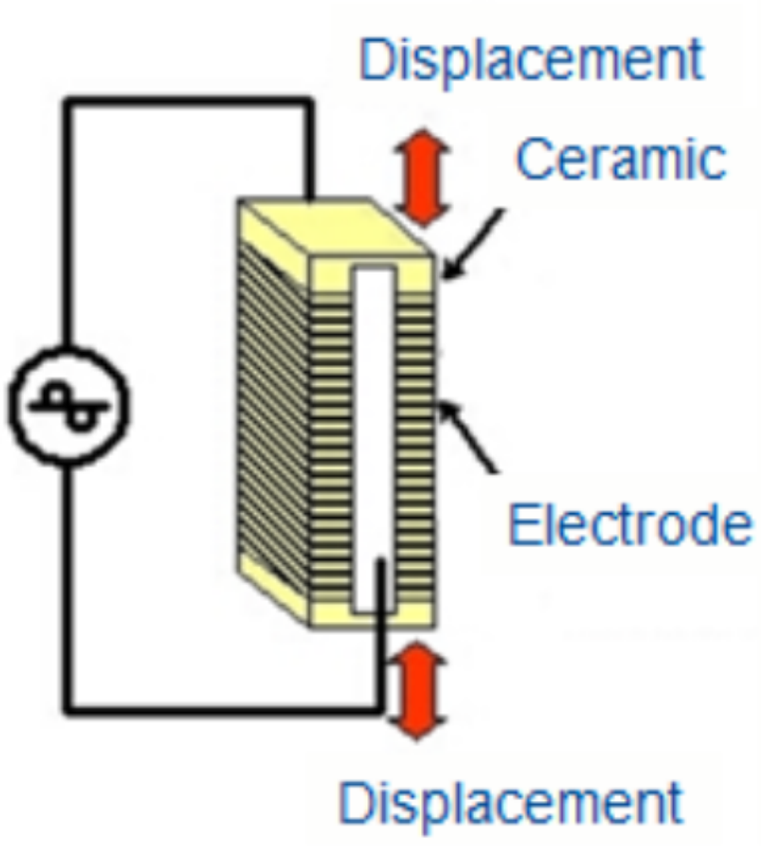


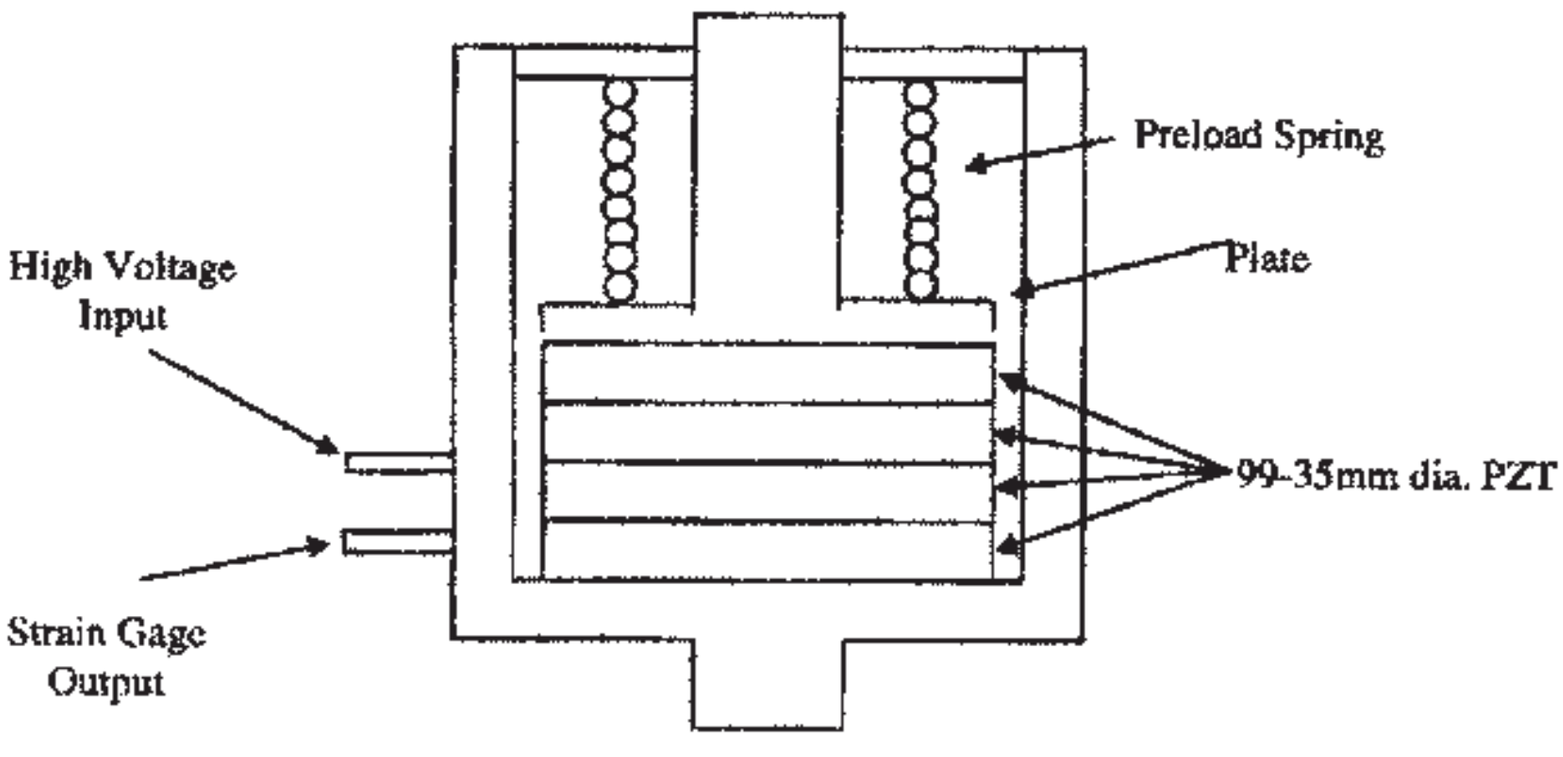
Usually a big screw, like doing it by hand but with a rotary motor

Hysteresis makes accuracy/repeatability impossible

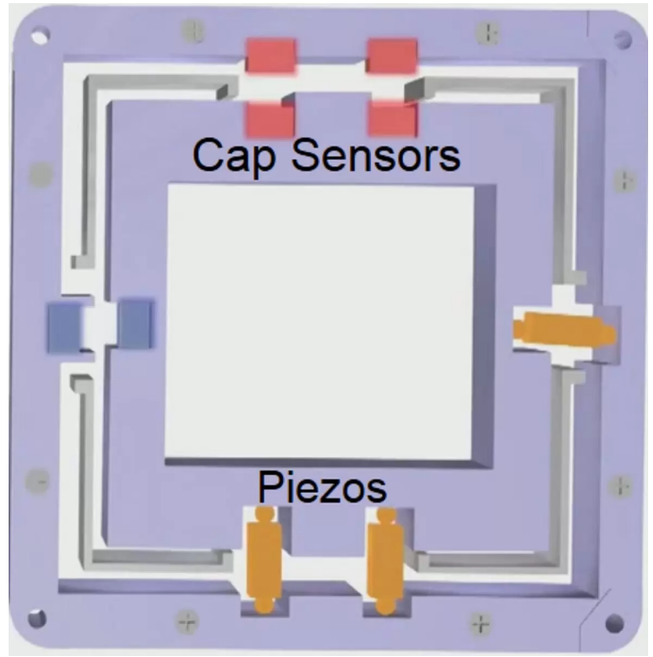
Sensors for closed-loop positioning

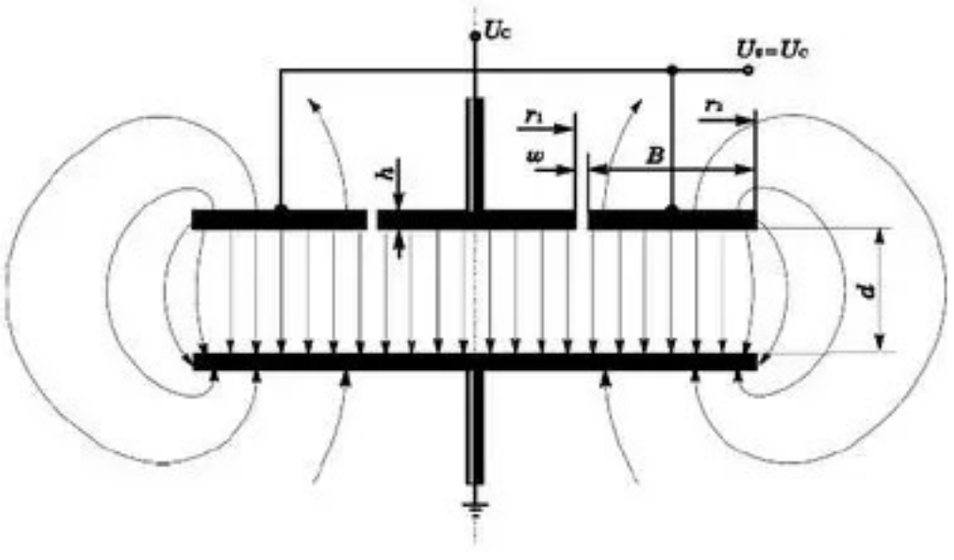




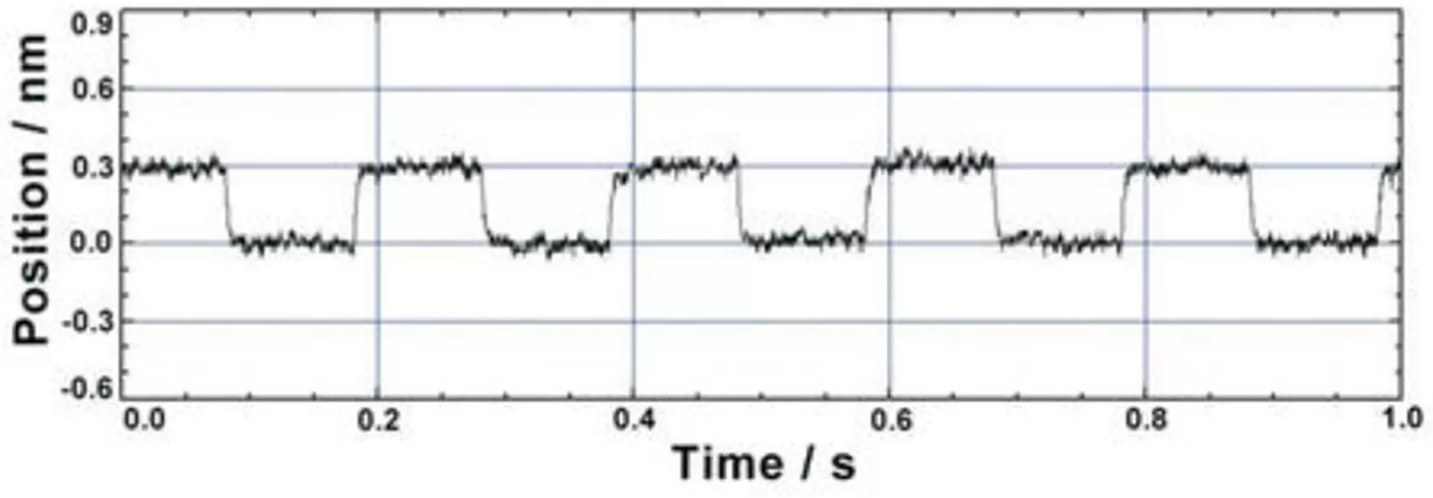


Sensors for closed-loop positioning

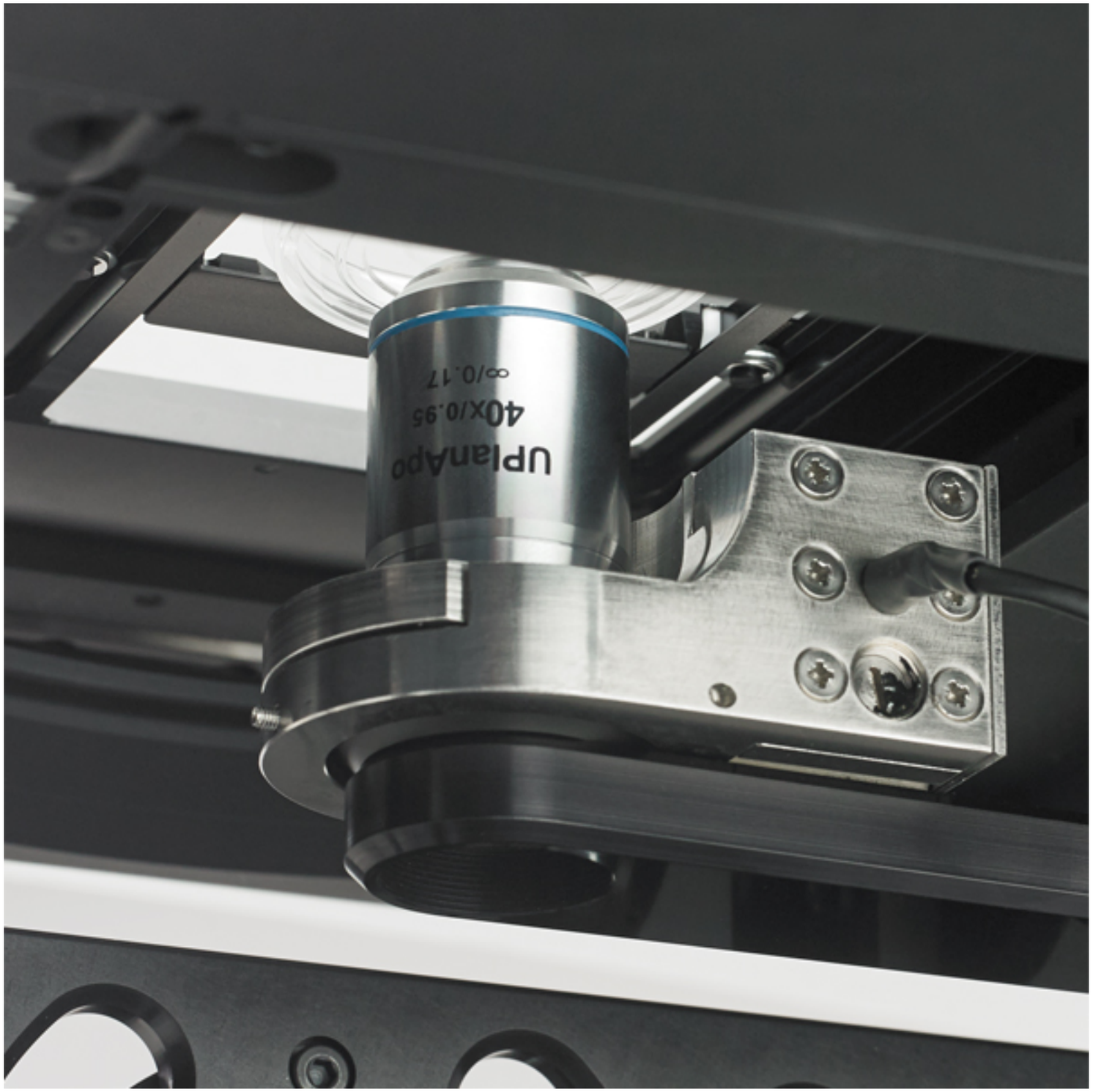


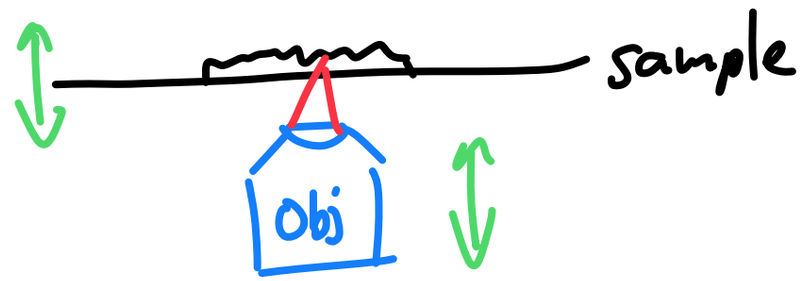


$$C \propto \frac{A}{d}$$



Piezo stages for objective (z) scanning





'Relative Position'