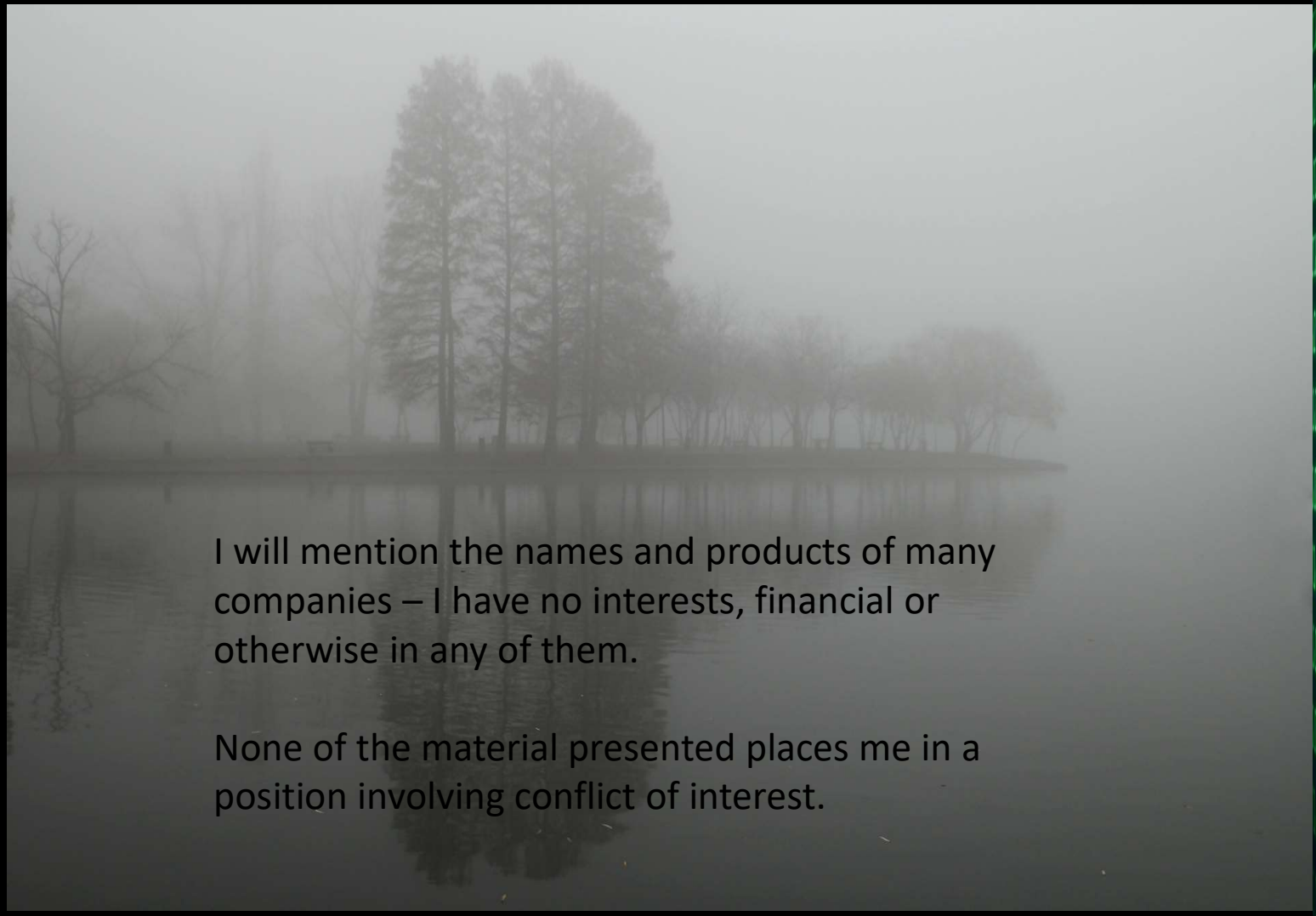
Digital Imaging in Pathology

- Past · Present · Future -



I will mention the names and products of many companies – I have no interests, financial or otherwise in any of them.

None of the material presented places me in a position involving conflict of interest.

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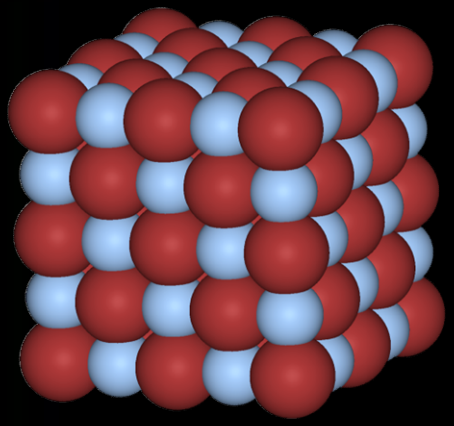
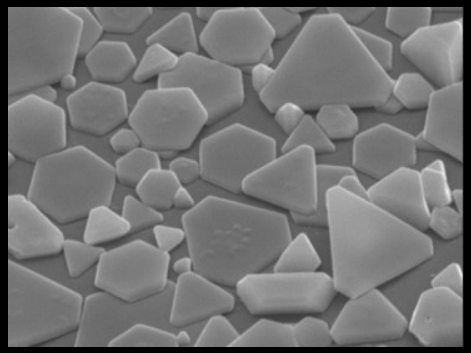
- Past · Present · Future -



Silver halide crystals form the basis of emulsion exposure photography



Silver halide crystals	0.2 - 2.0 microns
Resolution of crystals	6 - 10 microns
Grain (overlapping crystals)	10 - 30 microns (2540-847 dpi)
Human eye resolves	75 - 100 microns (340-254 dpi)

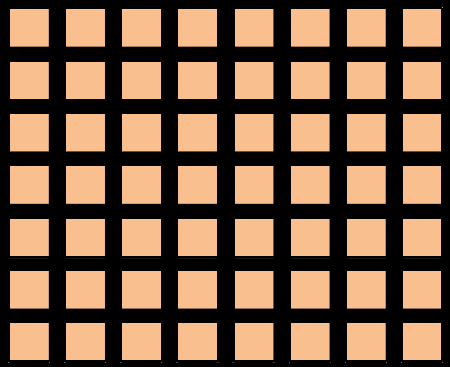
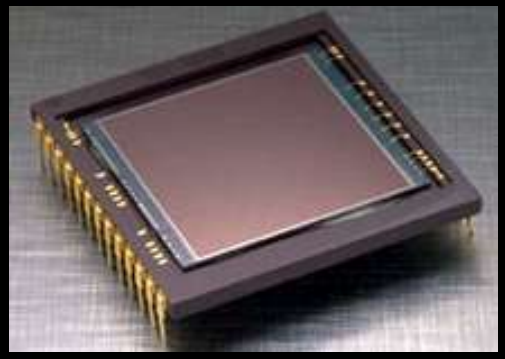
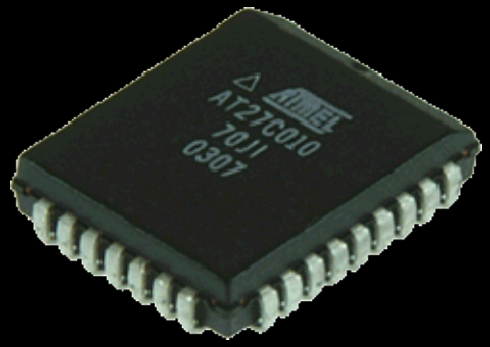


Photo sensor arrays:

- Sensitive to light, do not discriminate color.
- Two dimensional
- Electrical connections must be made (space between elements)
- Can be mechanically or electronically shuttered





Focus image on chip -------→ data moved ----------→ stored in memory

Cameras for still photography
- hand held cameras
- on light microscope
- on electron microscope

Digital video recording

Primary storage
- in the camera
- on a computer

Secondary storage
- on a disc
- in USB flash drive
- on a network drive

Acquisition ----→ Display

Data has quantity and quality in both acquisition and display

Key points that drove development of electronic devices:

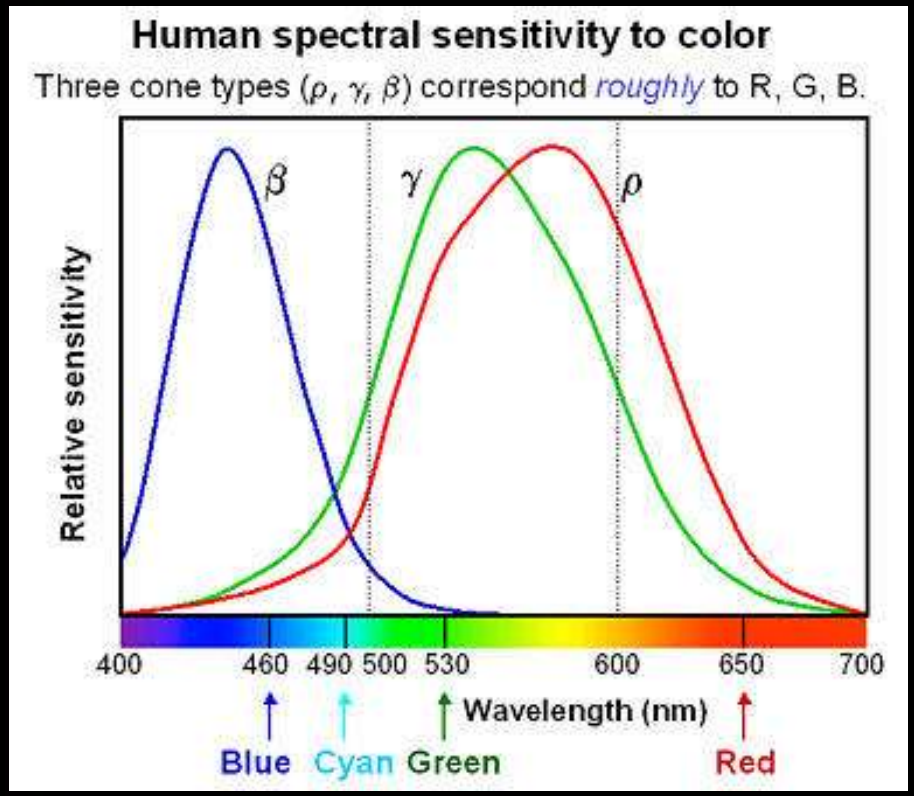
- Beyond your ability to perceive it, more dense data provides no visual advantage

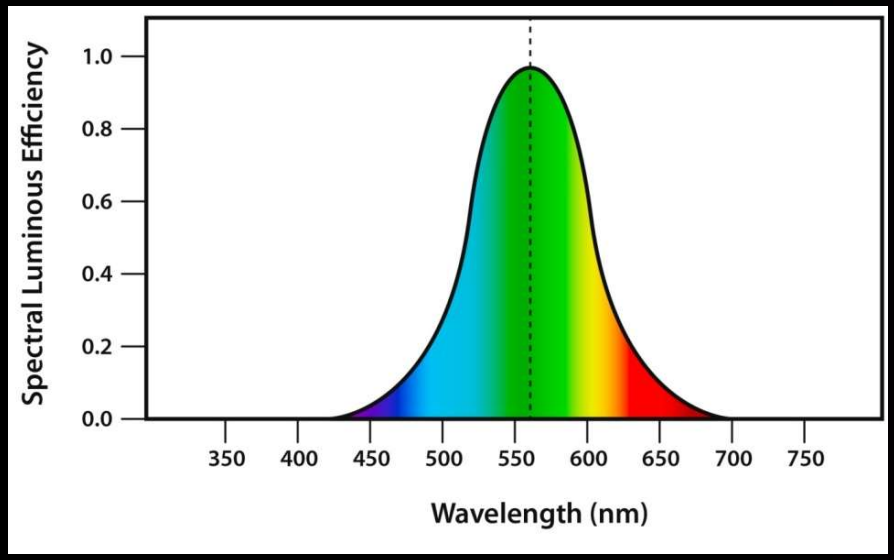
- It is an advantage if you want to zoom or blow up an image

- The evolution of digital imaging centered on increasing resolution and color fidelity

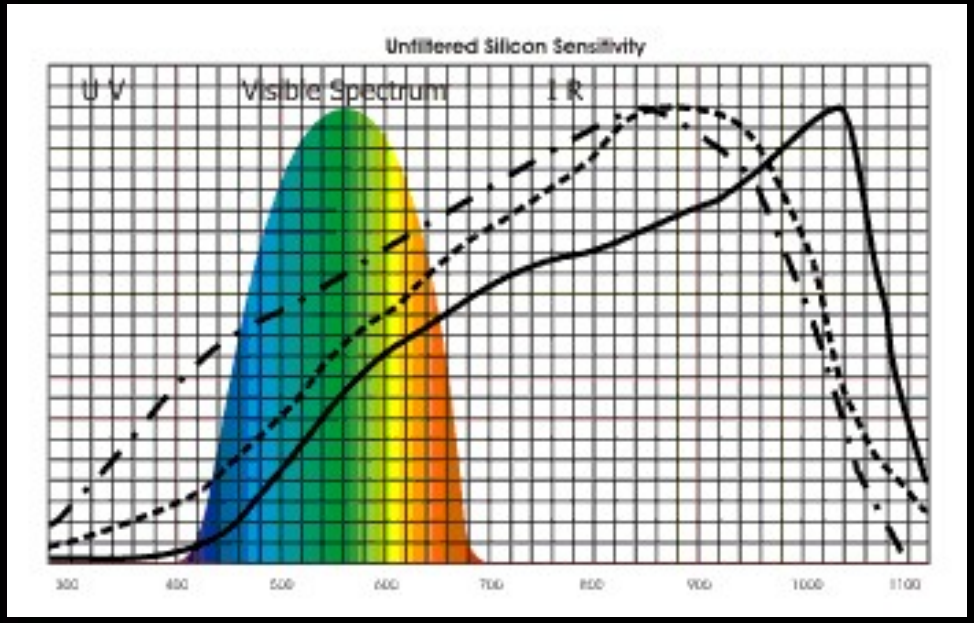
- You can throw away excess data; there is a limit to improving poor data.

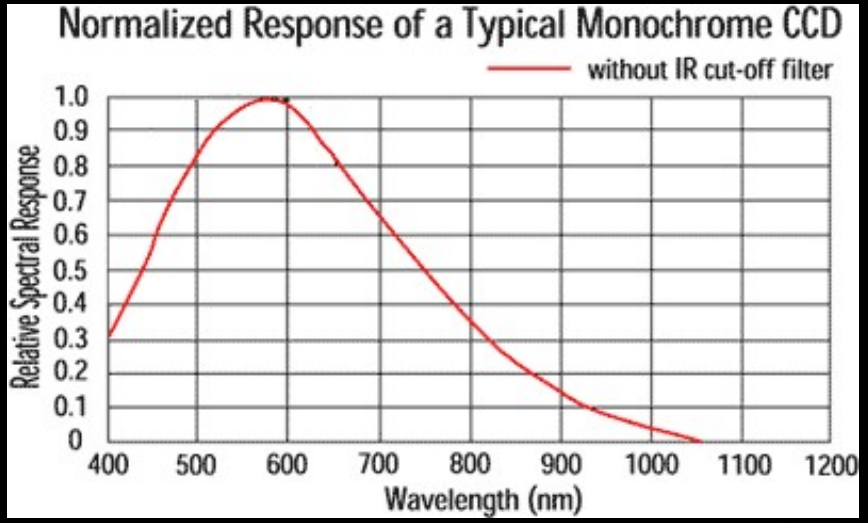
Spectral response of the human eye

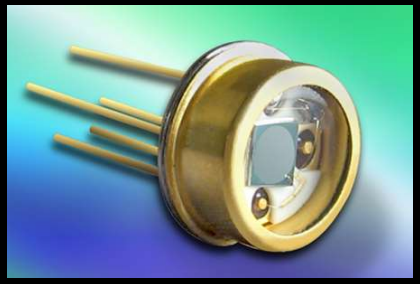


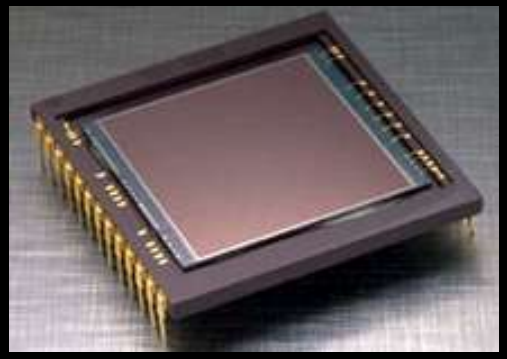


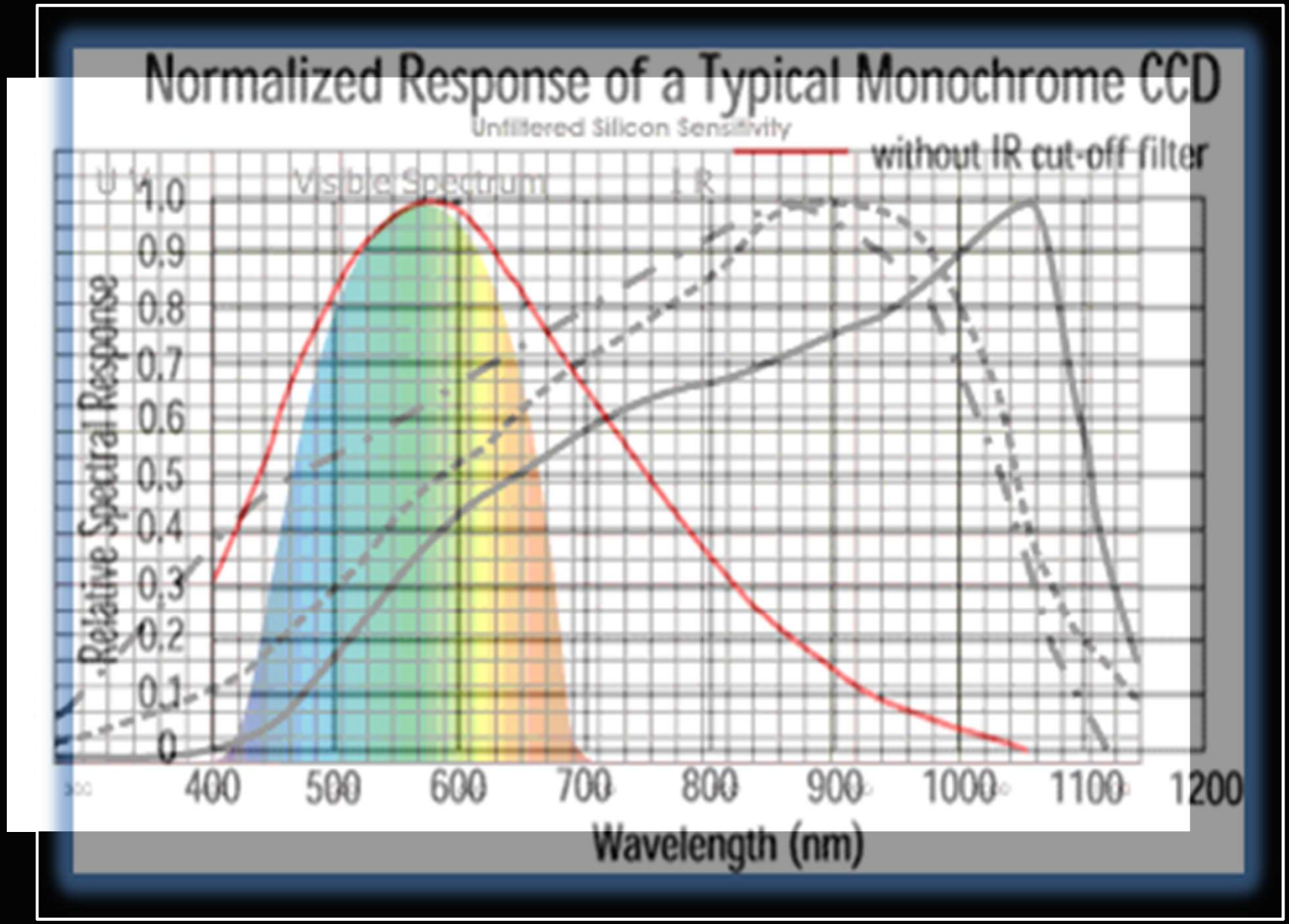
Spectral response of a silicon photodiode



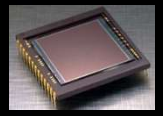







Normalized Response of a Typical Monochrome CCD
Unfiltered Silicon Sensitivity
without IR cut-off filter
Visible Spectrum
IR
Relative Spectral Response
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
400
500
600
700
800
900
1000
1100
1200
Wavelength (nm)

Shuttering



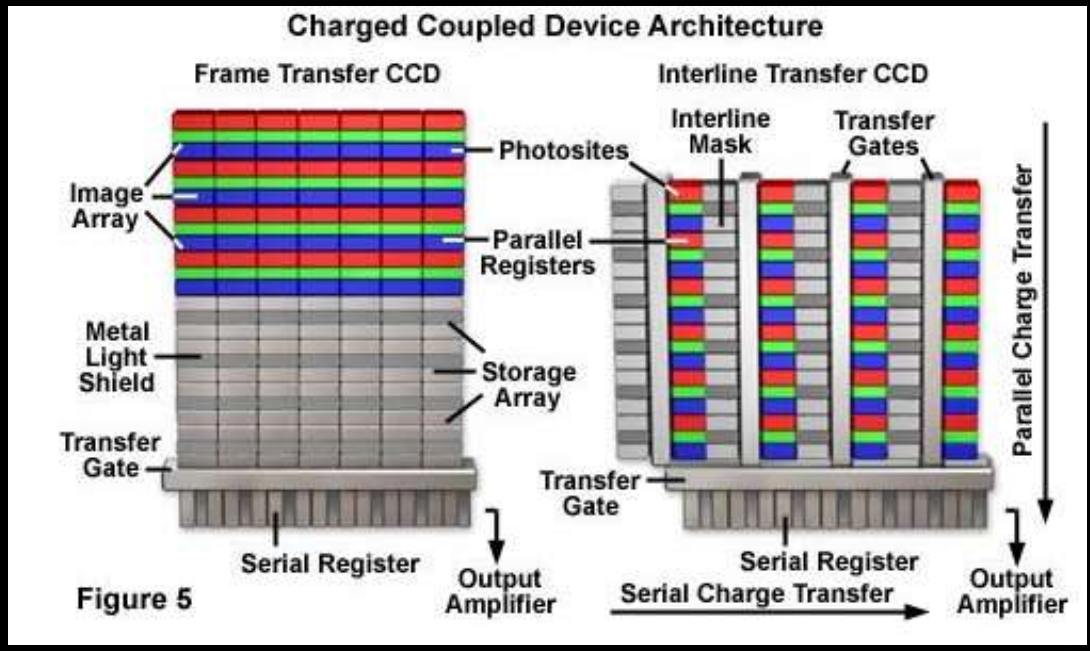
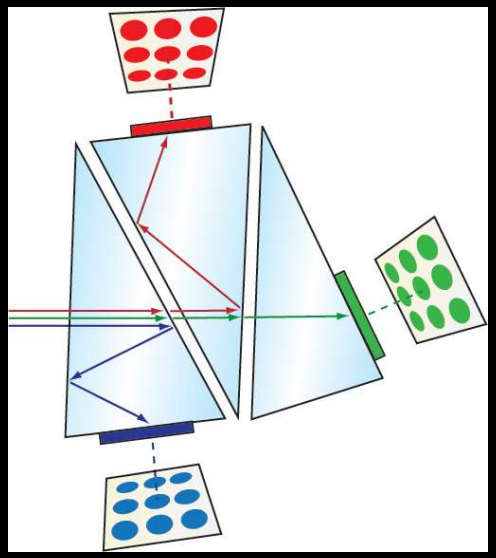
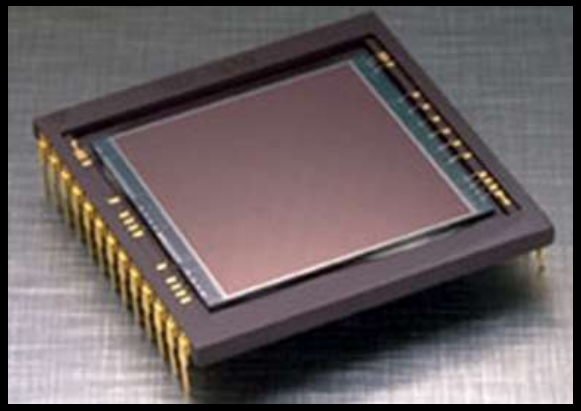


Figure 5

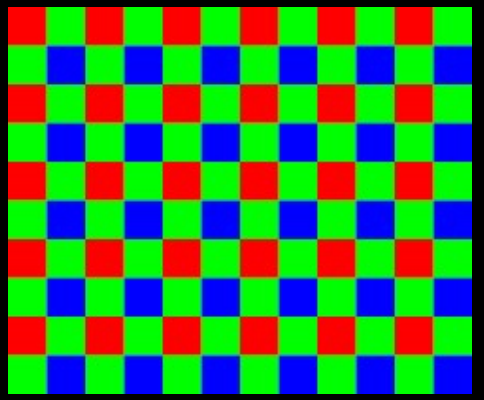
Adding Color



Prisms and 3 chips

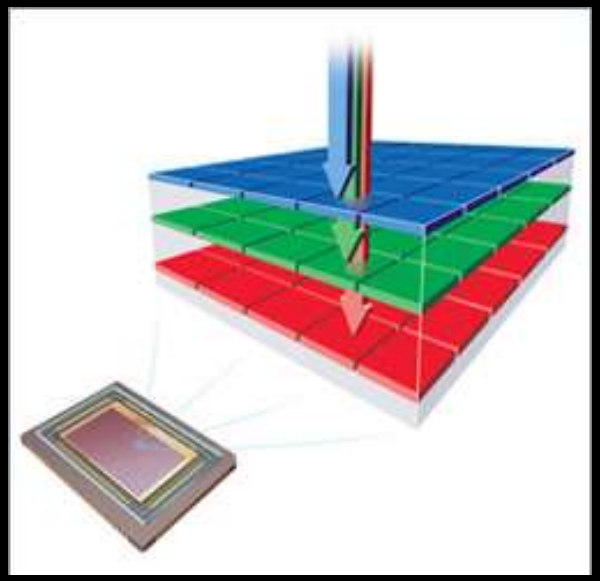


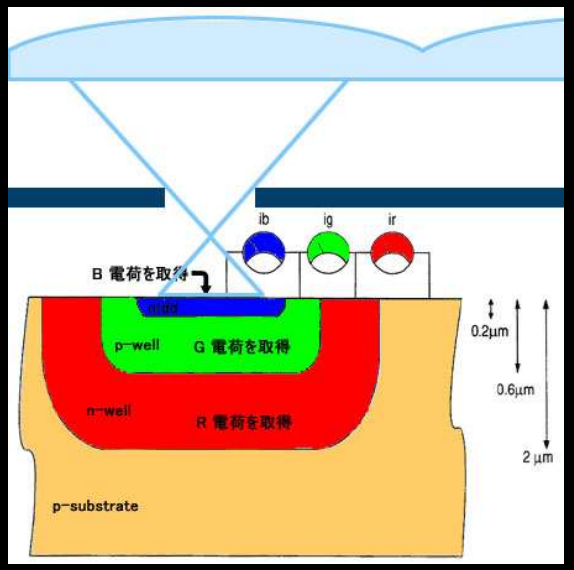
Filter wheel over
monochrome chip



Bayer mosaic filter over
monochrome chip

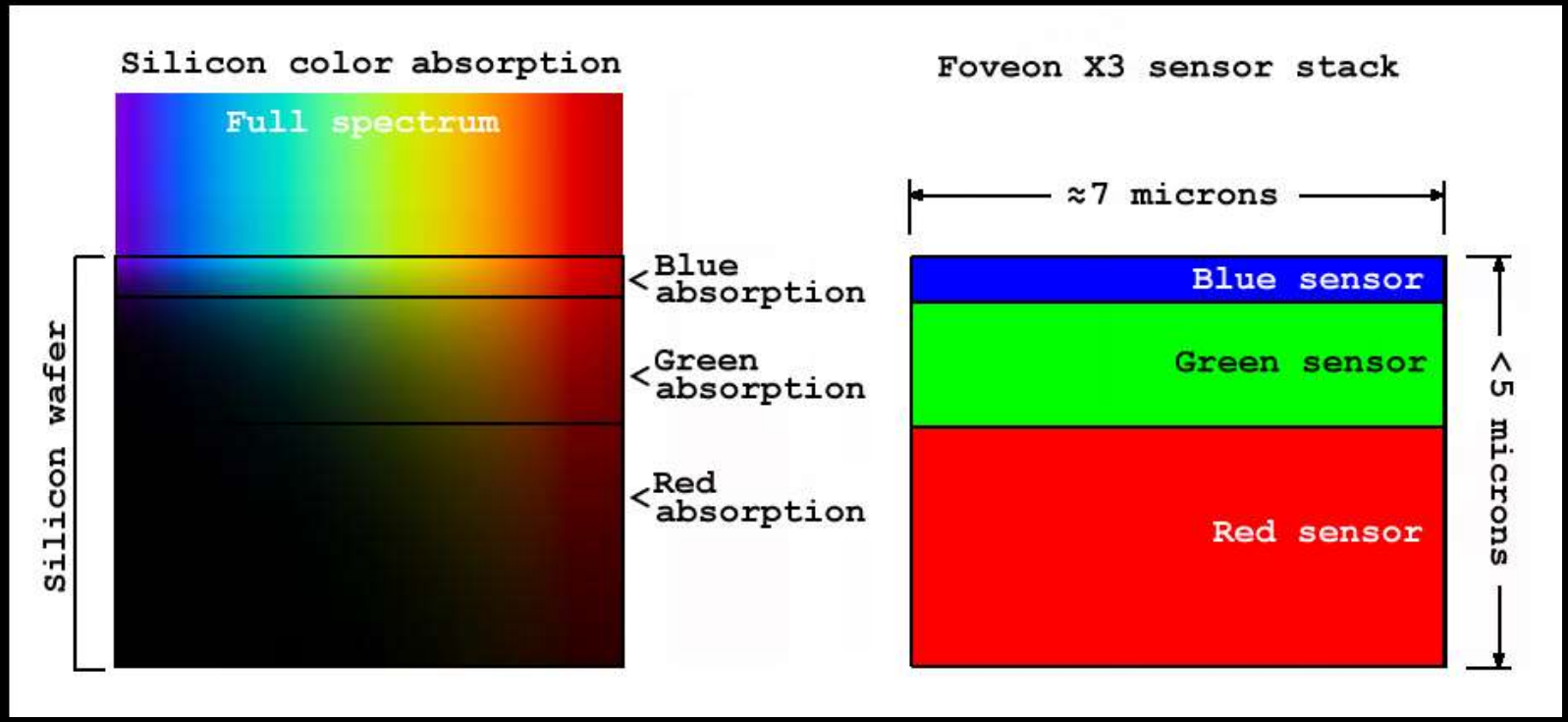
Foveon X3 Technology

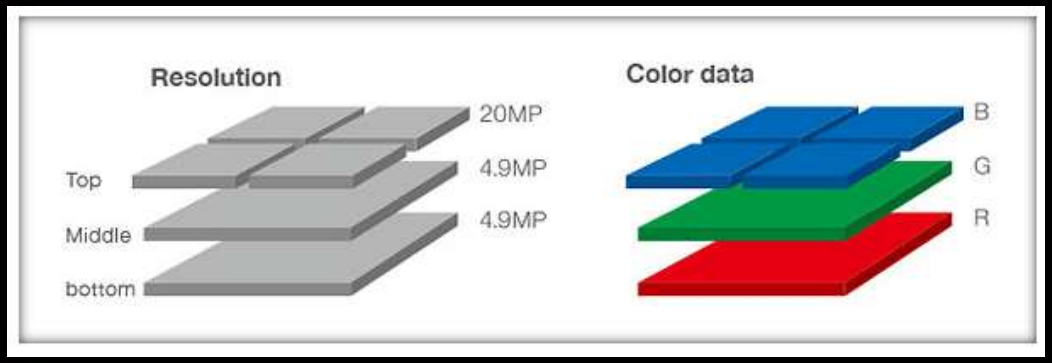




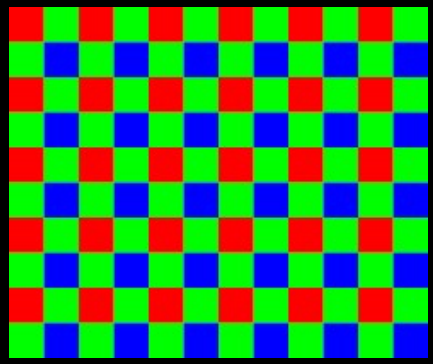
"A Dramatically Different Design

The revolutionary design of Foveon X3 direct image sensors features three layers of pixels. The layers are embedded in silicon to take advantage of the fact that red, green, and blue light penetrate silicon to different depths — forming the world's first direct image sensor"


Silicon color absorption
Full spectrum
Blue absorption
Green absorption
Red absorption
Silicon wafer
Foveon X3 sensor stack
≈7 microns
Blue sensor
Green sensor
Red sensor
<5 microns



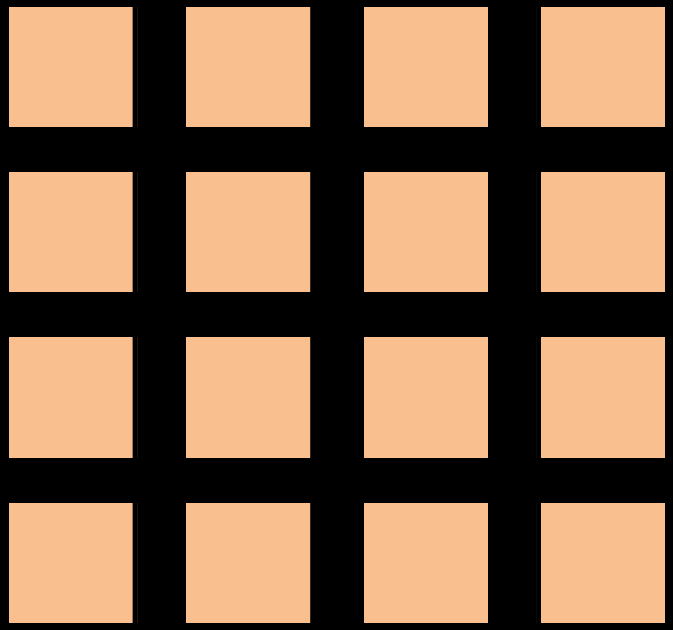
Foveolon



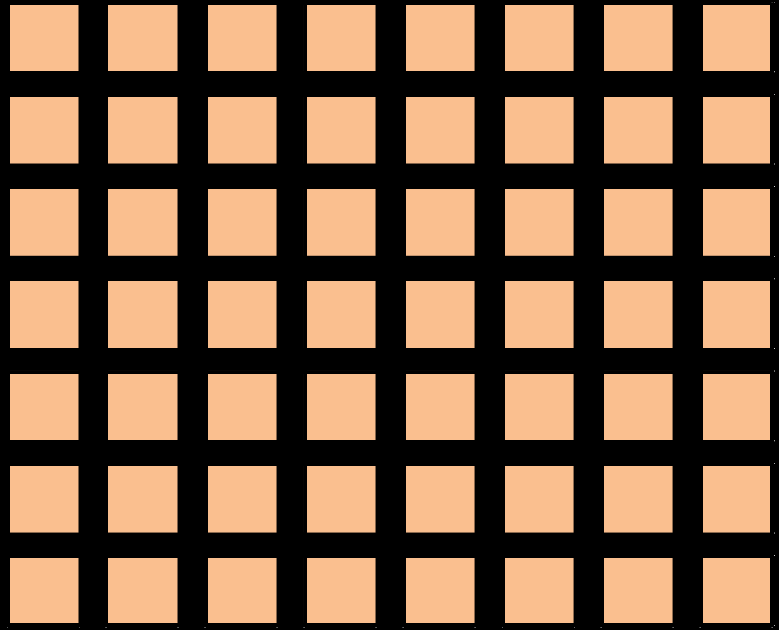
Bayer

- Foveon lacks color artifacts see with mosaic data.
- Foveon gathers more light overall, but elements respond less crisply to color and lower layers do not collect as well.
- Luminance is more difficult to measure with Foveon because blue is the wrong color to measure luminance. Bayer more accurately mimics human eye.
- Foveon has more chromic noise.
- Bayer has processing drain for interpolation, Foveon has processing drain to normalize the acquired data.

Pixel size big vs small



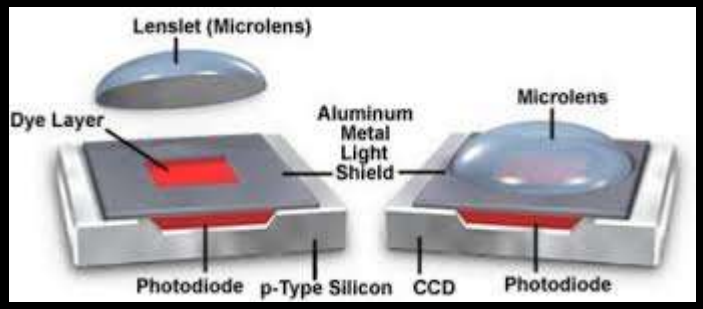
Big = better light gathering
= more sensitive.

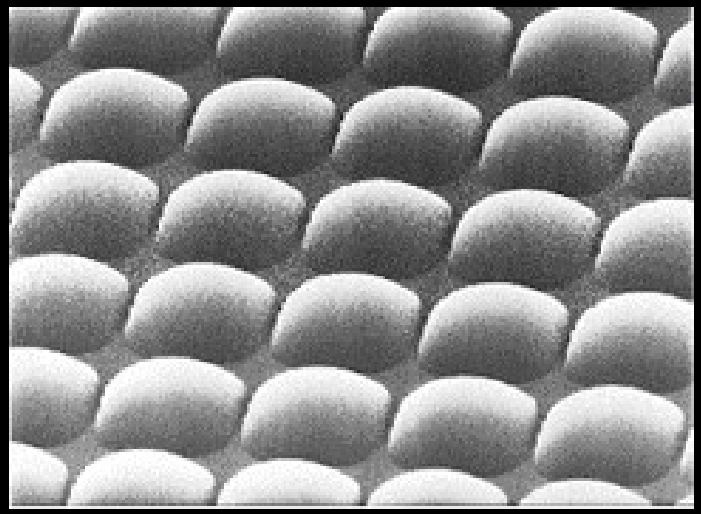


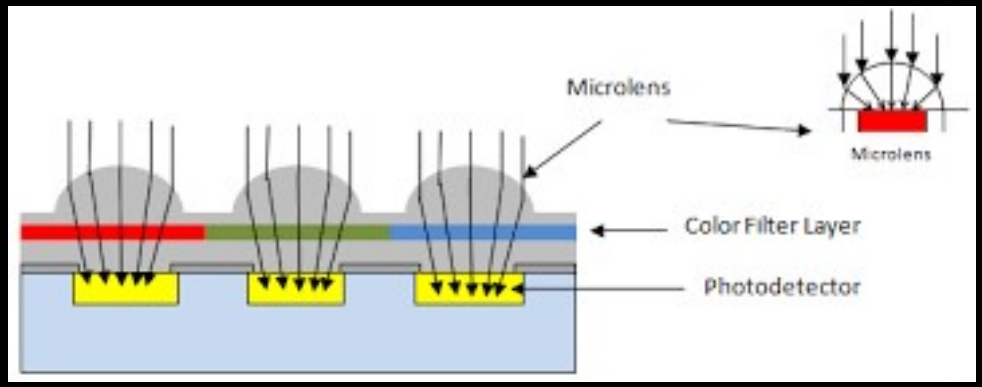
Greater number of pixels, less sensitive.

In a comparison of a triple chip camera using 1.3 megapixel chips and a single 5 megapixel chip, the 1.3 megapixel chips produced a better image.

Microlenses added for better light gathering

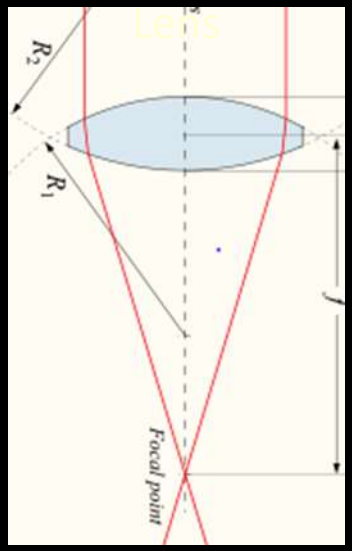


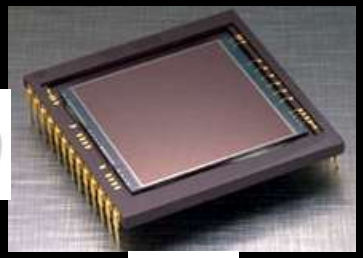


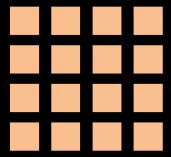


Physical collection of interpixel data

Progres 3012
Zeiss Axiocam
Olympus DP 70

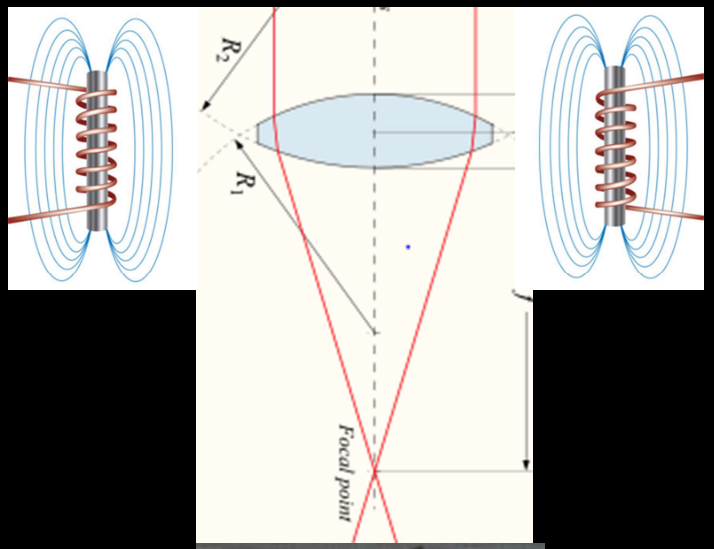


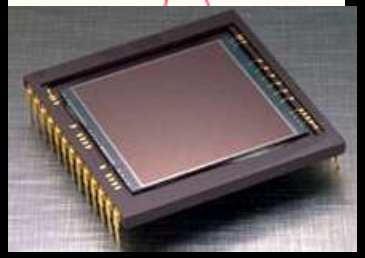


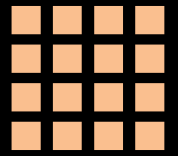


Pixera Penguin

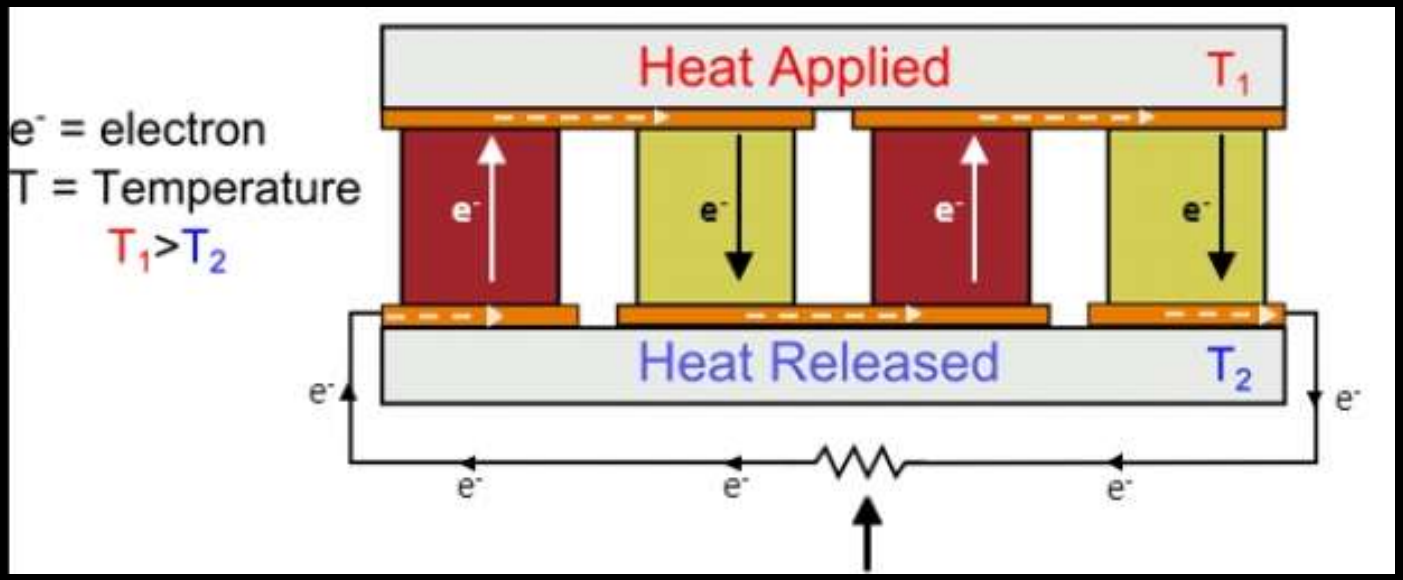
Ferromagnetic glass
Lens + Magnets

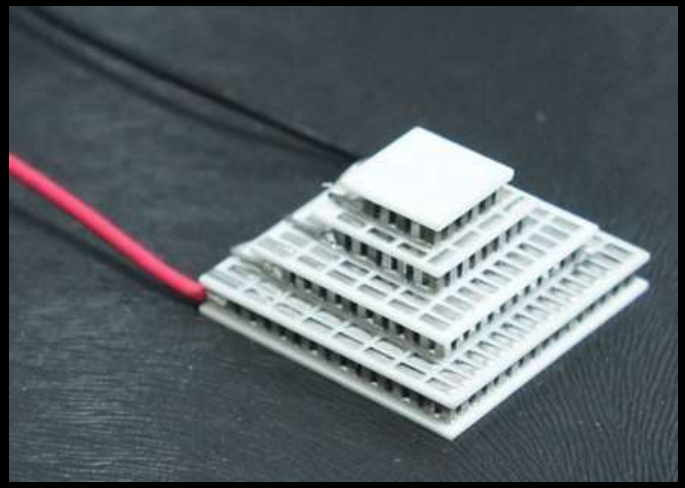






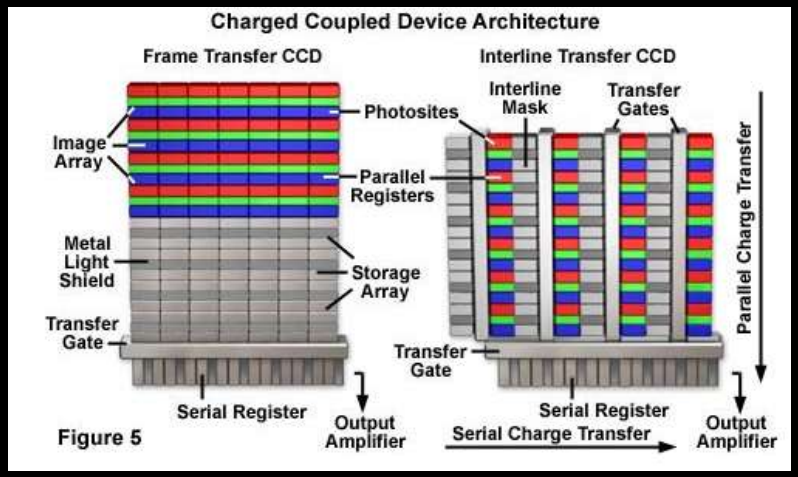
Chip Cooling – Reduces Noise
Peltier Thermoelectric Device

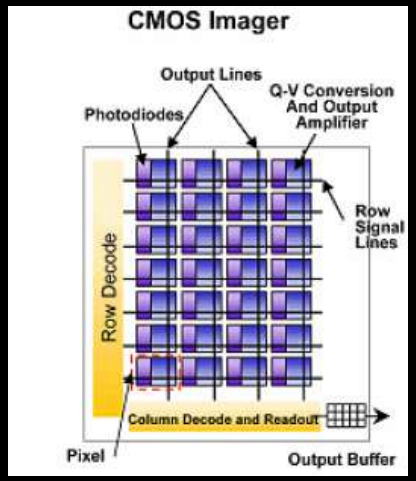




CMOS Sensors

Complimentary metal oxide silicon



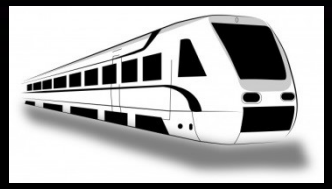


CCD – off sensor processing

CMOS – on sensor processing

CMOS Sensors:

- Fundamentally different in construction and operation.
- Made of same type of silicon as many solid state control devices so amplifiers, for example, can be formed on same silicon as sensors. Power consumption less, less heat, less noise.
- X-Y addressing to acquire data directly, makes them fast.



Train has arrived

50MP 43.8 x 32.9mm
CMOS sensor

Kodak:
1975 – First digital camera 100x100 pixel
1988 – First megapixel camera

- resolution and color fidelity has reached a point that the consumer will give up film.

- the benefits of being able to analyze, manipulate, and use the image surpasses film in countless ways.

- image acquisition and sophisticated processing are now in the hands of the individual.

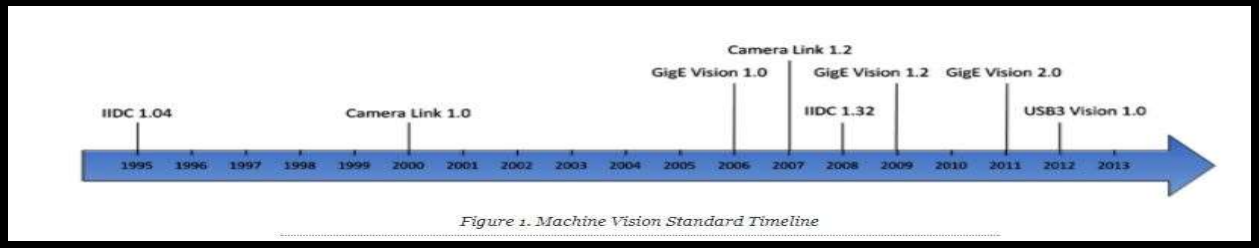


Figure 1. Machine Vision Standard Timeline

Firewire 1995 Camera Link 2000 Gig E 2006 USB-3 2012

Still shot quality reached level to replace film

High Quality Hand Held Cameras
large chips
change lenses

Photo Stand
cooled CCD
piezo shift or large chip
motorized Zoom Lens

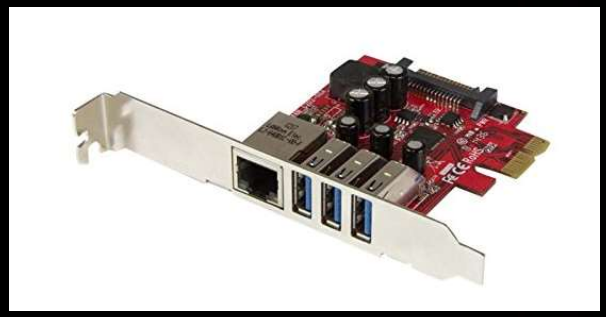
Microscope Cameras
cooled CCD
piezo shift or large chip

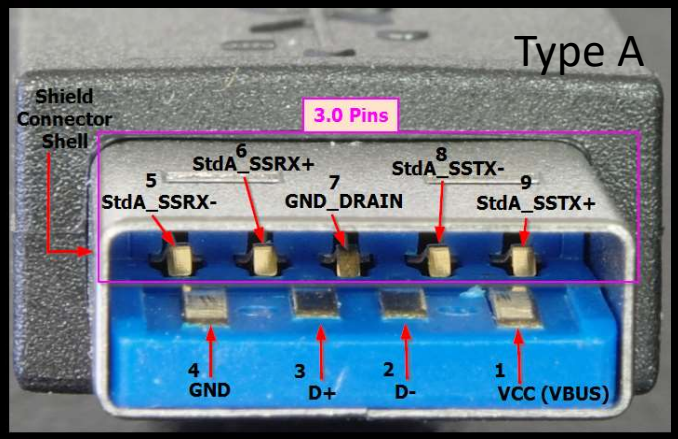
Developments after pixel shift

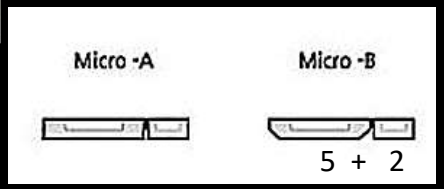
Fast transfer:

- **Gig-E** gigabit transfer over a network cable
- **USB-3** (high speed USB, 5->10->20 Gbps)
  (3.0, 3.1, 3.2 or 3.1 Gen1, 2,3)

RJ-45







Type A

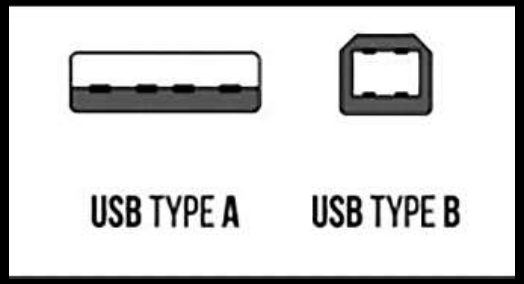
Type B

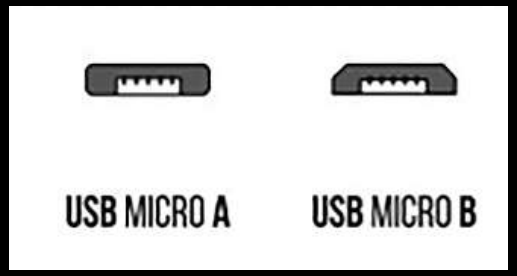
USB 2 USB 3

USB-2
Micro-B

USB2
Mini-B

Type A – plugs into computer or charger





USB 3

USB 2

USB2 4 pin

USB3 7 pin

USB3 7 pin – with screw connector

usually supplied with camera

Type B – plugs into peripheral device (printer, cell phone)

RJ-45

Ethernet Cables – Patch Cables – LAN Cables

- not "crossed cable"
- all have RJ-45 connectors , not part of specification.

First choice is: C2G C2G.com , cablestogo.com

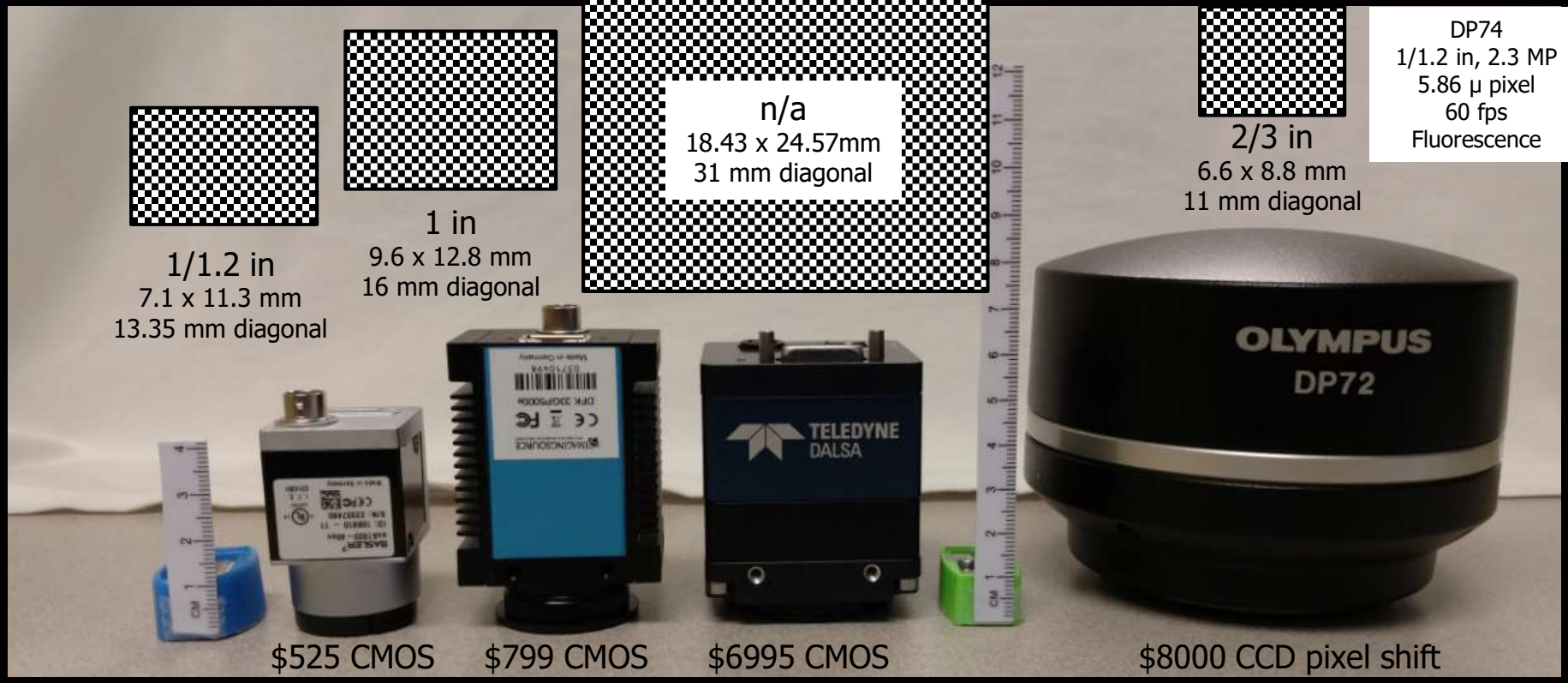
Cat 5e	1 Gigabit to 100 meters	8 ft - $5.99 USD
Cat 6	3 Gigabit to 100 meters	8 ft - $8.99
Cat 6	10 Gigabit to 55 meters	
Cat 6a	10 Gigabit to 100 meters	8 ft - $15.99

Cable options (Cat 6, 8 foot):

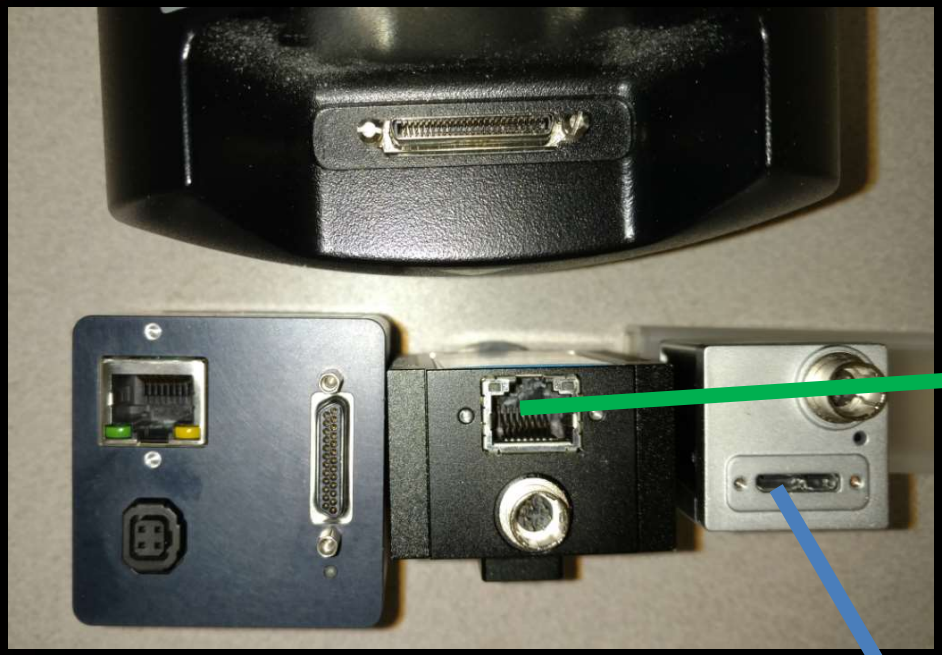
- Snagless Patch Cable	$ 8.99
- Shielded Patch Cable	$11.99 Electromagnetic and Radio frequency interference (EMI/RFI)
- Non-booted Patch Cable	$ 8.99
- Slim Patch Cable	$10.99
- Plenum Patch Cable	$35.99 (7 ft) in air ducts, low smoke, low flame
- TAA Snagless Patch Cable	$10.99, 13.99 (7, 10 ft) TAA gov standard
- Crossover Patch Cables	- do not use -

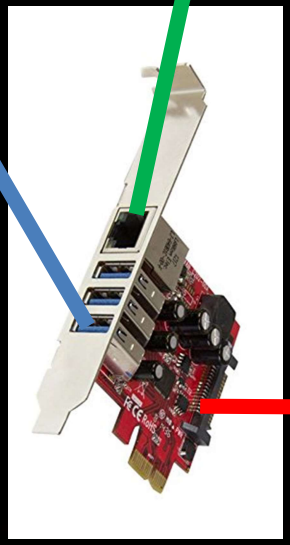
10 colors

Gray Blue Black Green Red Yellow Orange Purple White Pink



1. Basler, acA1920-40uc	2.3 MP (1920x1200)	5.86 µ pixel	41 fps	global shutter
2. Imaging Source, DFK 33GP5000e	5.3 MP (2592x2048)	4.8 µ pixel	22 fps	global shutter
3. Dalsa, Genie TS-C4096	12.6 MP (4096x3072)	6.0 µ pixel	12 fps	global shutter
4. Olympus DP72	1.36 MP (1360x1024) 3.2 MP (2070x15470 12.8 MP (4140x3096)	6.45 µ pixel pixel shift pixel shift	15 fps	progressive scan





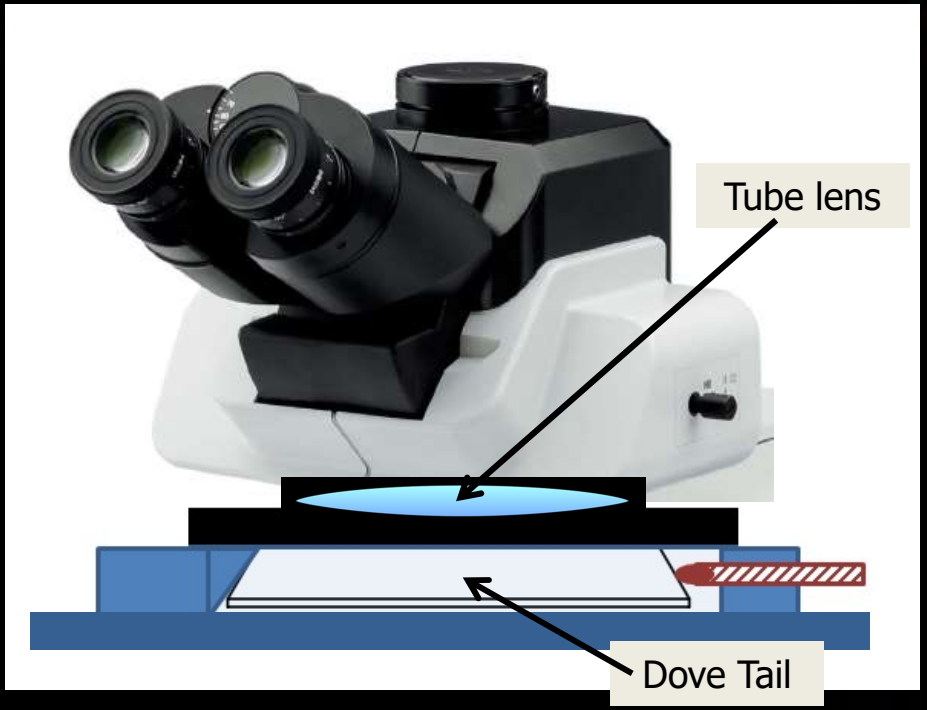
Sata Power Cable

All are C-Mount, 25.4 mm opening except
Dalsa Genie has a 42 mm opening

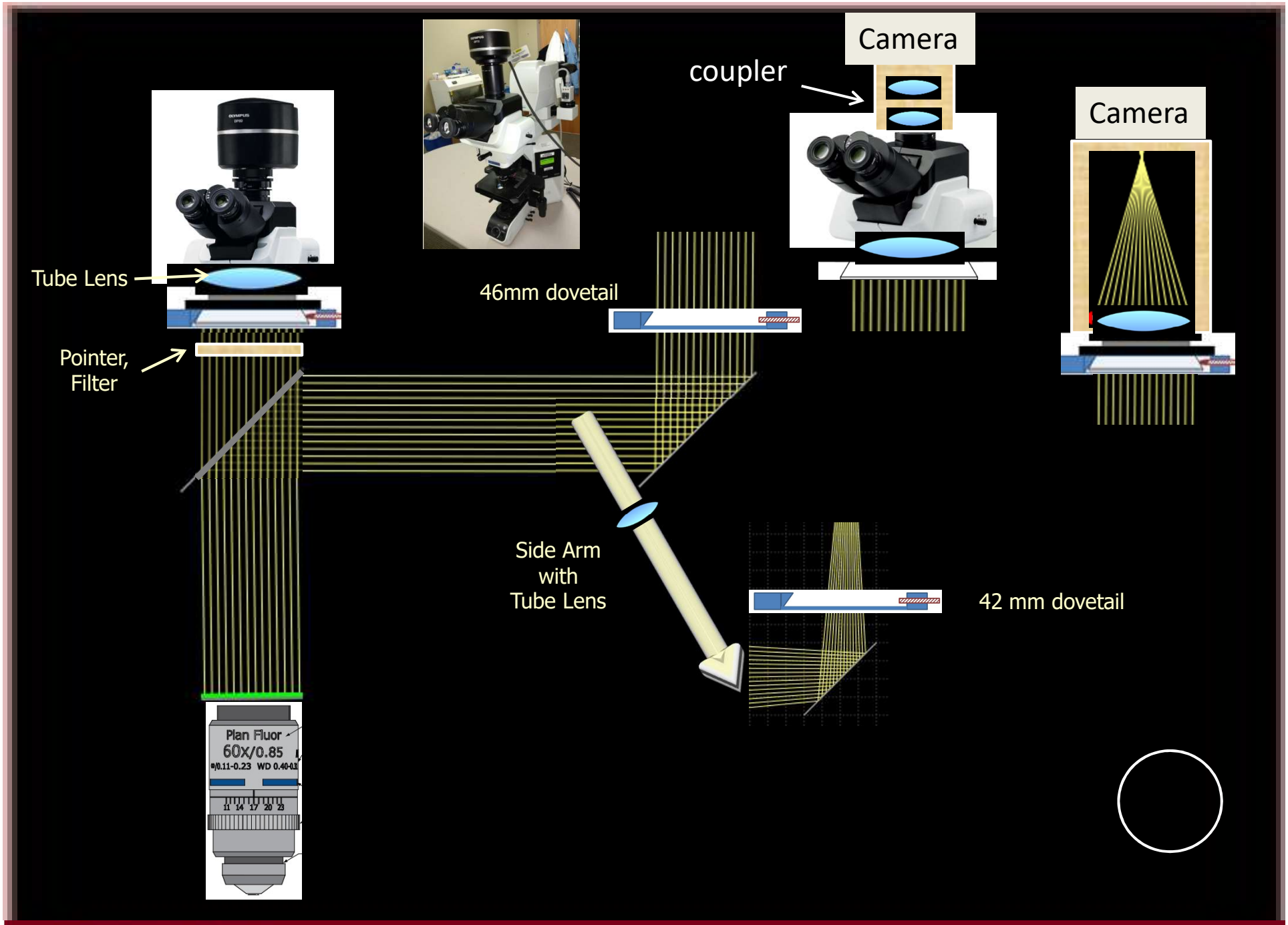
Olympus BX53

Stock couplers for:
0.35 x … 1/3 inch format
0.50 x … 1/2 inch format
0.63 x … 2/3 inch format
1.0 x … no optics


OLYMPUS
DP72



Dove Tail Connection


Camera
coupler
Camera
Tube Lens
46mm dovetail
Pointer,
Filter
Side Arm
with
Tube Lens
42 mm dovetail
Plan Fluor
60X/0.85

Edmund Optical:
C-mount 1 inch x 32
T-Mount M42 x 0.75

ThorLabs:
SM1 1.035 inch x 40
SM2 2.035 inch x 40

Dalsa Genie: M42 x1 (Pentax thread)

- Thor has extensive line of thread adapters.
- Thor has mounted lenses.
- Edmund has more lenses of some types but you will have to mount them and accommodate their lens mount geometry.

RAFCAMERA

http://www.rafcamera.com

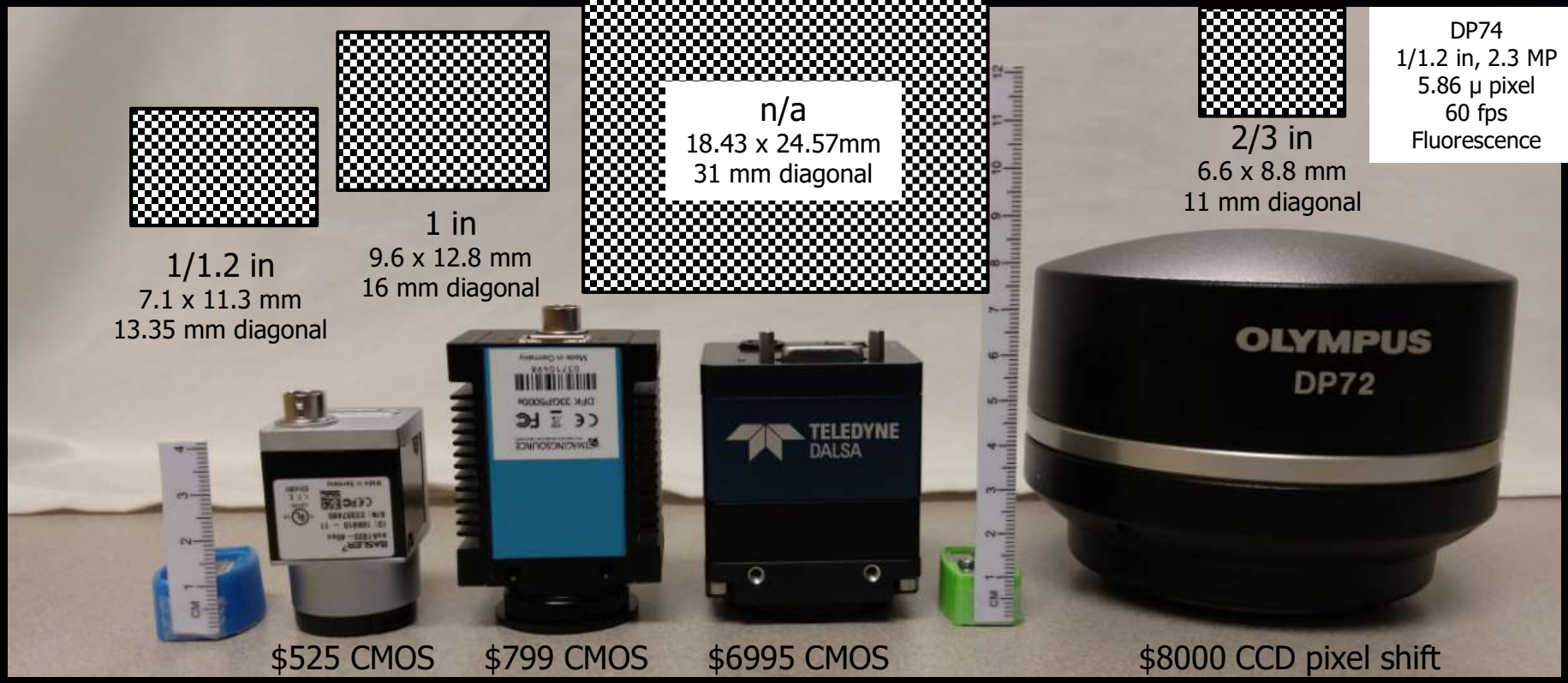
Company based in Belarus

Machine shop produces custom adapters (example: dove tail to C-mount) on demand in quantities of 1 or more.

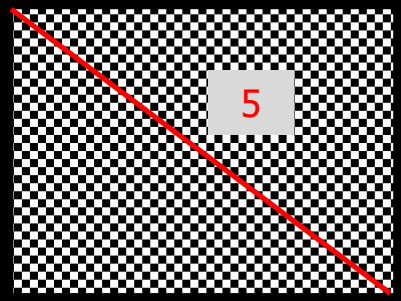
Price $30 USD

Turn around:

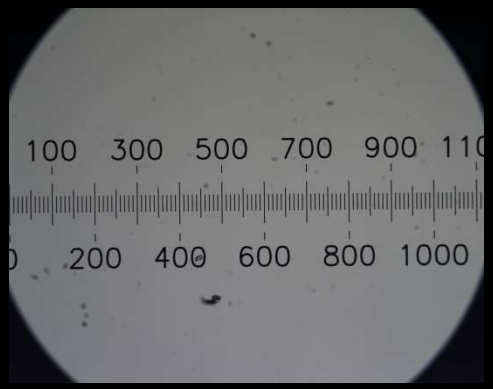
- Scale drawing for your approval (days).
- Batches to anodize the machined tempered aluminum about 1 per week
- Finished product within 2 weeks.
- Mail, express from Belarus, 1-2 weeks.
- Overall turn around, under 4 weeks.



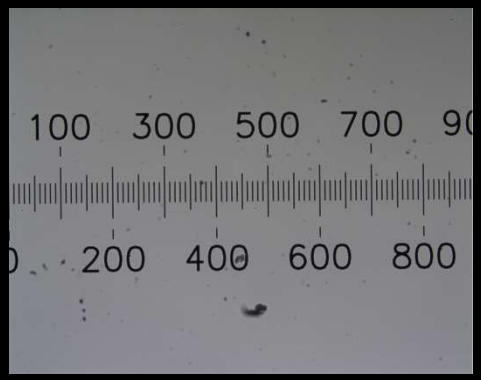
Aspect ratio 1:1.333



#2 f-100, 11.15 mm



#2 f-125, 8.95 mm, 80% of available width, 64% of area.

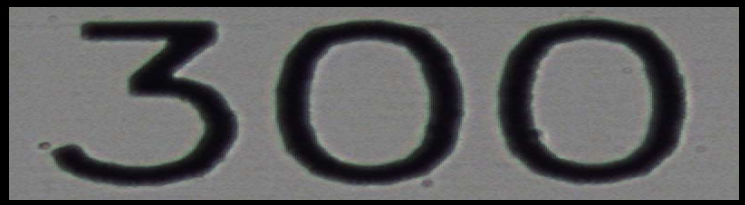


1. Basler, acA1920-40uc	2.3 MP (1920x1200)	5.86 µ pixel	41 fps global shutter
2. Imaging Source, DFK 33GP5000e	5.3 MP (2592x2048)	4.8 µ pixel	22 fps global shutter
3. Dalsa, Genie TS-C4096	12.6 MP (4096x3072)	6.0 µ pixel	12 fps global shutter
4. Olympus DP72	1.36 MP (1360x1024) 3.2 MP (2070x15470 12.8 MP (4140x3096)	6.45 µ pixel pixel shift pixel shift	15 fps progressive scan

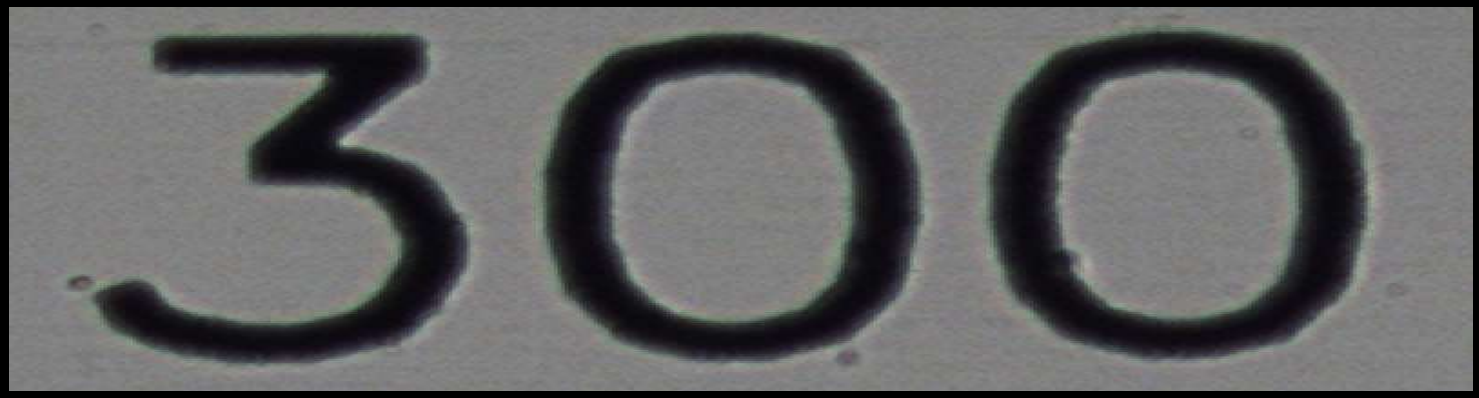
Performance:

Imaging Source	36 x 28 inch pixelate from 100% to 200% . 11.4 MB tiff . fov 890 microns /f125
Dalsa	56 x 42 inch pixelate from 100% to 200% . 36.9 MB tif . fov 880 microns /f250
Olympus	38 x 29 inch pixelate from 66.7% to 100 % . 38.1 MB tif . fov 712 microns /f ?

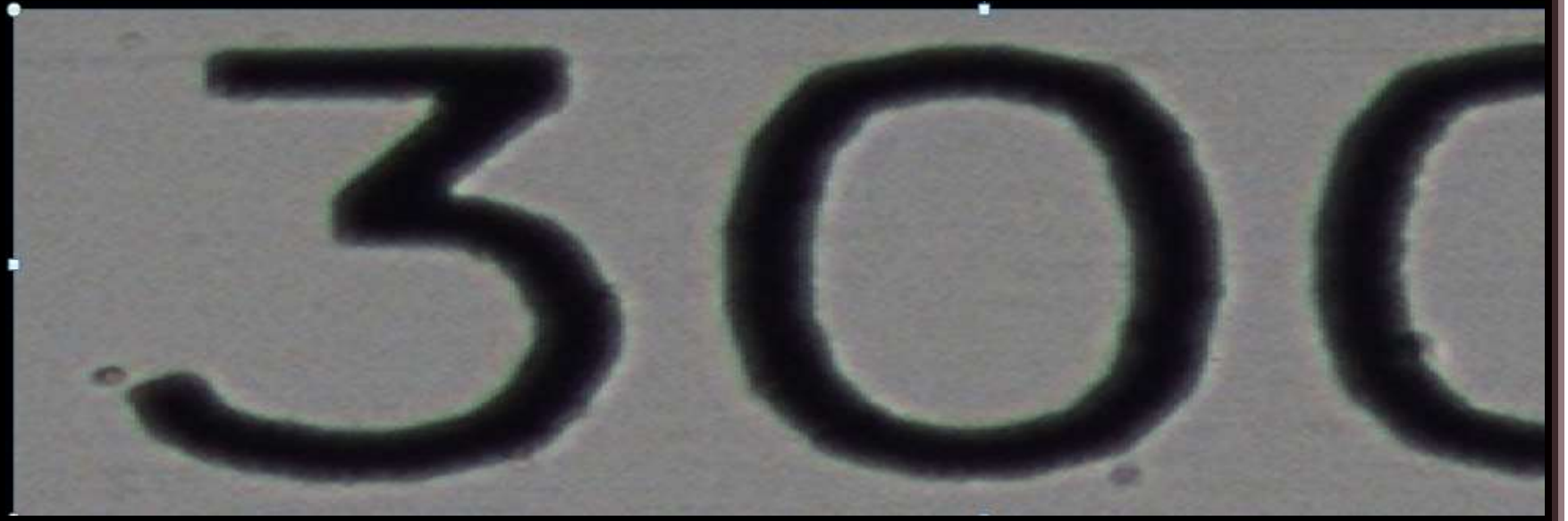
1X



2X



3X



Imaging Source: Microscope Camera (DFK MKU130-10x22)

"Achieving and maintaining sharp focus in microscopy can be a challenging task. The Imaging Source 13 megapixel microscope camera has been designed to be a cost-effective, versatile solution for demanding microscopy applications. Featuring Sony CMOS technology, this camera comes equipped with a distortion-free autofocus lens which allows viewers to capture exactly what they see through the ocular rather than being limited to a region of interest. This camera can take the place of the ocular itself or can be screwed into the C-mount - eliminating the need for costly adapters. The camera's USB 3.0 interface makes a full HD preview (at 30 fps) on the host PC a reality".

Resolution: 4128 x 3096 (12.8 MP)

Shutter: Rolling

Frame rate: 30 fps @ $\leq$ 1920 x 1080; 1 fps @ higher res.

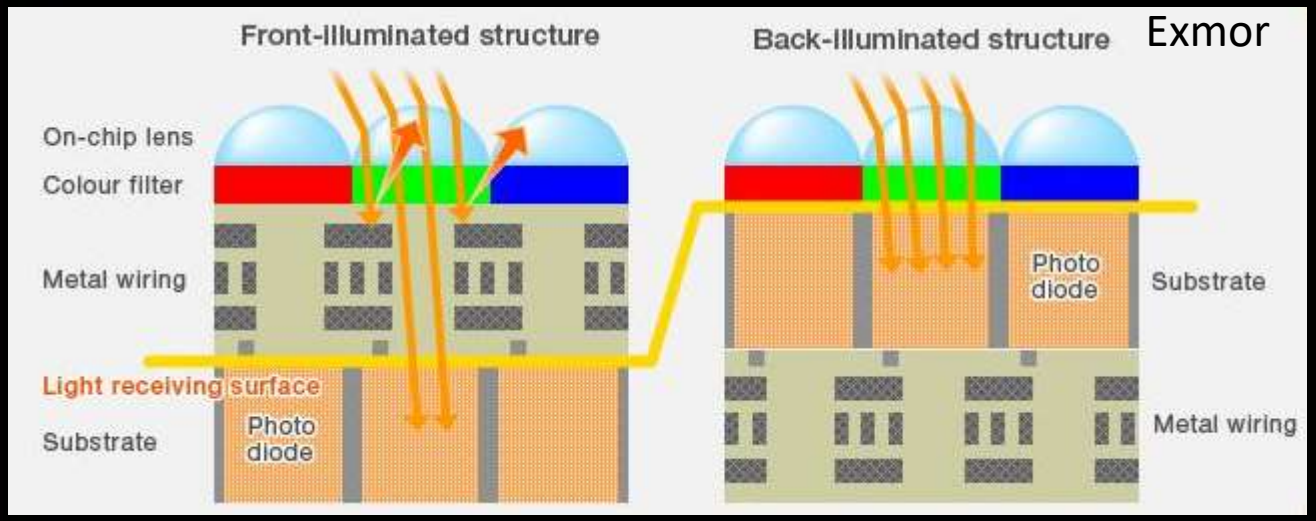
Sensor : CMOS Exmor

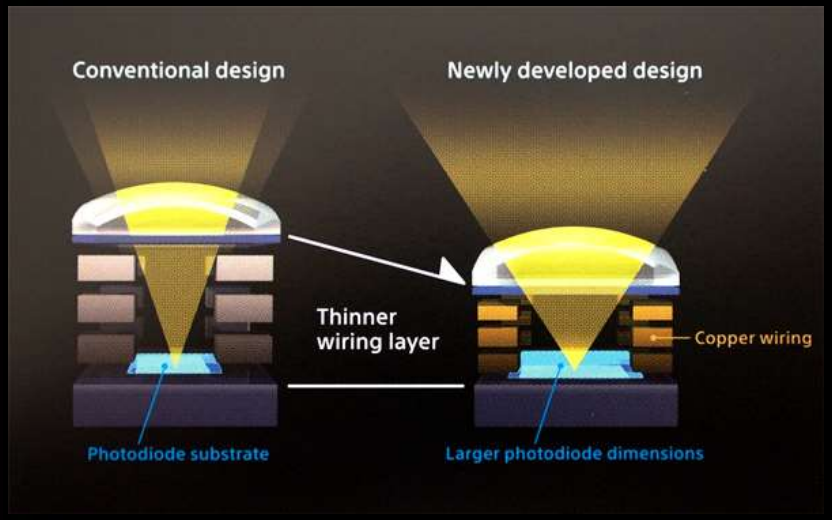
Format: 1/2.5 (4.3 x 5.8 mm; 7.2 mm diagonal)

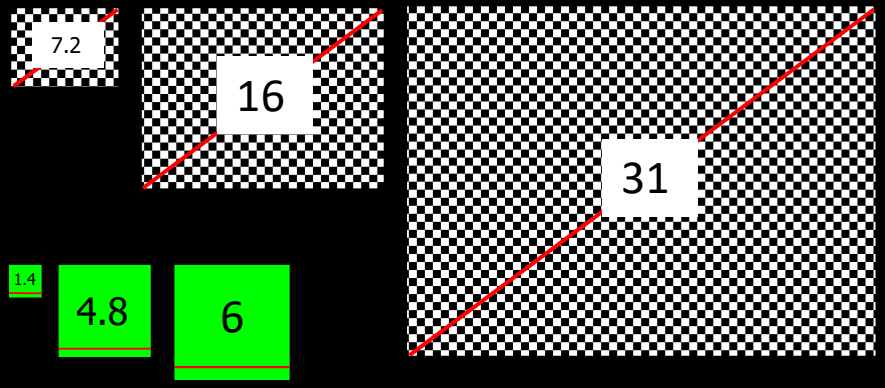
Pixels: 1.4 microns

Imaging Source 2592x2048 ($1.4^2/4.8^2$) = 8.5% Dalsa Genie 4096x3072 ($1.4^2/6^2$) = 5.4%

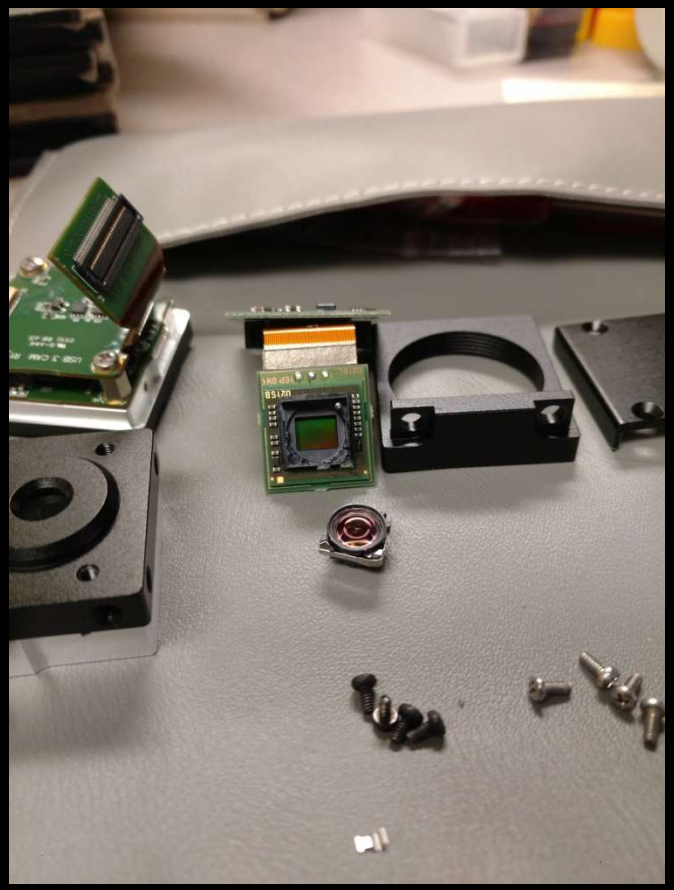
Pixel improvements:








0
300
200
400




Corporate Express

Whole Slide Imaging - WSI

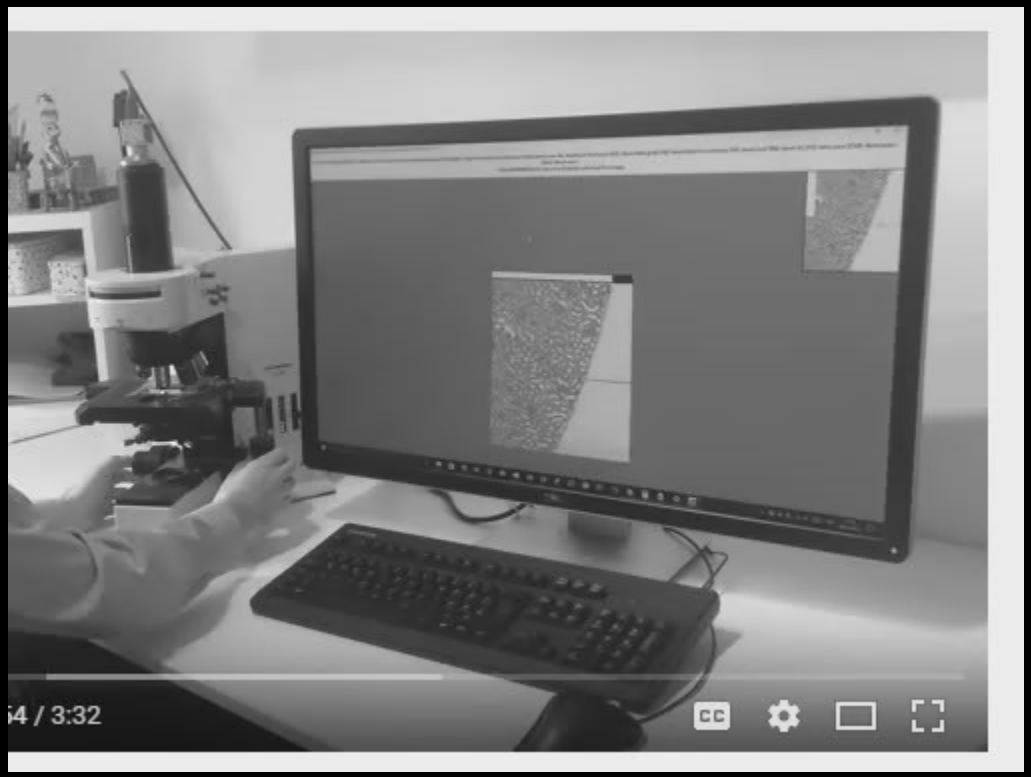
1. Load slide in above tray and insert in machine.
2. Machine previews slide and picks multiple focus points (or user may select the points).
3. Once machine is satisfied it begins scan and saves image to server.
4. Pathologist accesses server and examines image.

- Not all images scan well or at all (machine may reject).

- Telepathology is used as a backup (video camera connected to a video server, accessible on high speed lan).


eSlideManager - Table - Internet Explorer
http://lisa-esm/Records_Li
eSlideManager - Table
Sort
View Images
Open Data
Copy
Assign To
Column 1-5 of 11
Clone To
Export Data
Annotations
Label
Image
Captured Date ↓
ScanScope ID
Status
Analysis Progress
WEST QA
2017/11/16
08:40:54
LEICA-ESM1
East Bank
DAILY QA
2017/11/16
08:18:53
LEICA-ESM2
DAILY QA
2017/11/16
07:50:33
LEICA-ESM3
3mm
RUO
19800 x 12364 x 3 = 700MB, File = 52MB
-28 , -4097 : 19800 x 12364
8706 , -2305
prefetching / trackmap / progressive rendering

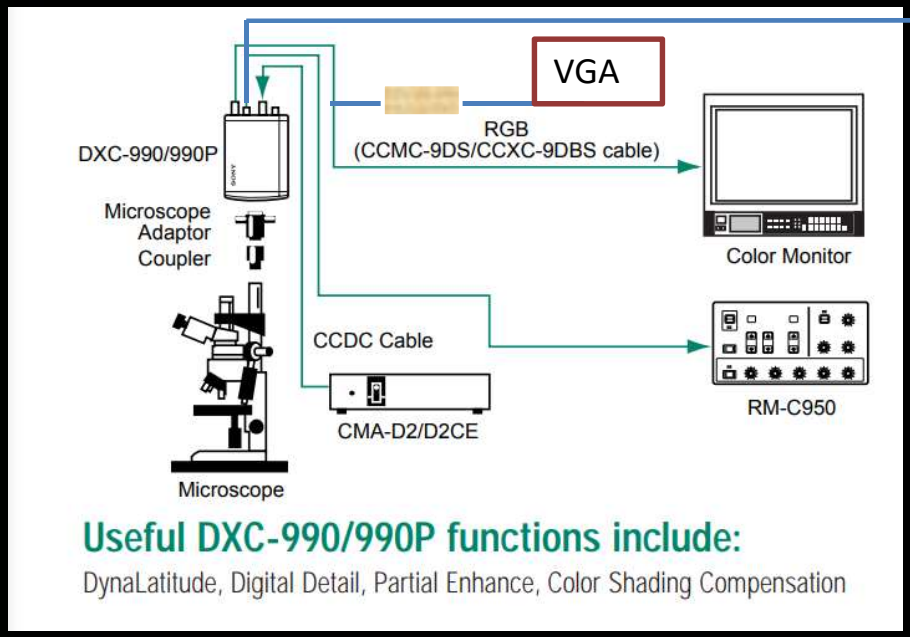
Manual Scan with “Microvisioneer”



- Install was quick and easy
- Drivers install with an executable.
- Camera specified lists for $525.
- Also need a USB-3 card and means to couple a C-mount camera to your microscope.
- Software is $1989 for education and research, $2989 for commercial use.
- Save as tif or svs, plenty of freeware viewers.

www.microvisioneer.com

Outputs:
- Composite video
- S-Video
- RGB Video



AXIS COMMUNICATIONS

Video In – Digital Out
Fast compression in hardware

Network Camera – Read Specs

Shutter	Rolling Shutter
Max. Image Circle	1/2.5"
Sensor Type	CMOS
Resolution (H x V)	2560 px x 1920 px
Resolution	5 MP
Pixel Size (H x V)	2.2 µm x 2.2 µm
Frame Rate MJPEG	9 fps
Frame Rate MPEG-4	9 fps
Frame Rate H.264	9 fps

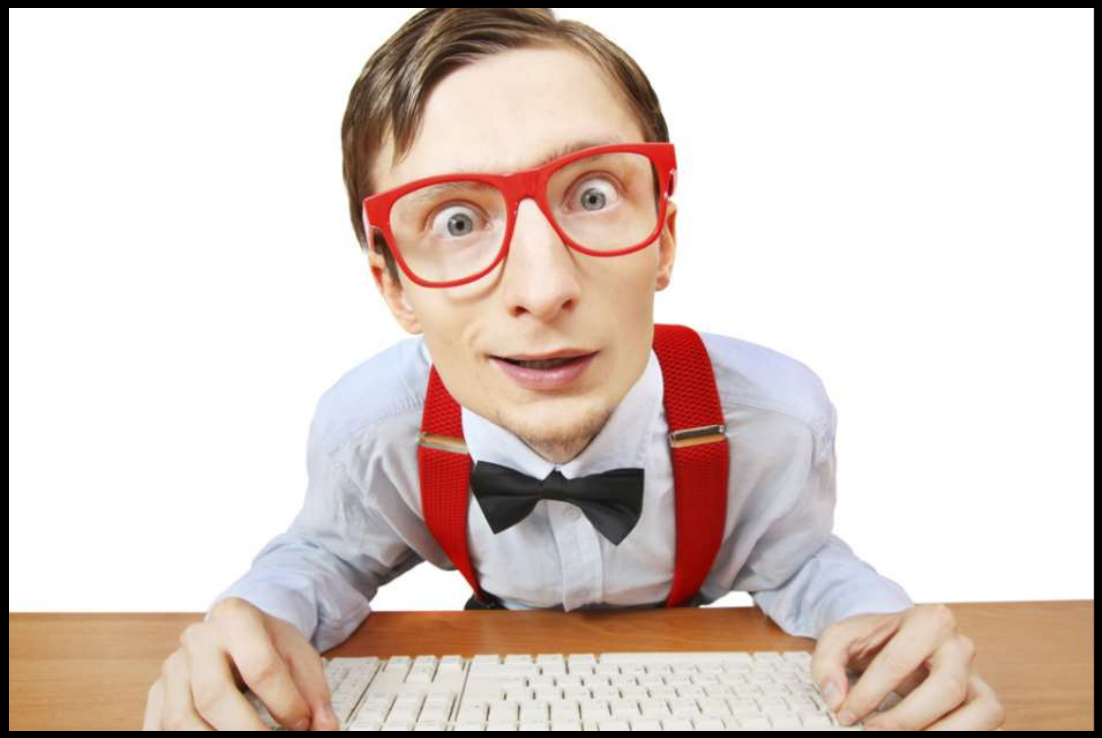
Connectors	▪ RJ-45 connector for 10/100 BASE-T Ethernet, full or half duplex

Gig-E

- Compact package that can serve as a video and still shot camera
- Robust sensors that compete with pixel shift
- Imaging Source camera ships with easy to use software.
- Dalsa software is very granular but at the developer and not user end.
- Output on a network cable but not serving video or web media.
- Exploring the possibility of remote viewing through VPN.
- Streaming possible via software server.

- Define the mission – what do you need.
- Consider those you serve, talk to them.
- Discuss plans with others that have already deployed systems.
- Get the computer services department involved. You will need someplace safe to store your images.
- Be careful how you compromise, people grumble when you ask for more but quickly quiet when a new service runs well. They forget slowly when there is a big expenditure that produces a failed system.

Hire an Informatics Professional



Stereotype

Actual group of Informatics Professionals


plugable
USB 2.0
Fast Ethernet


ACT
LINK

Mark Luquette, M.D.

LUQUE007@UMN.EDU

Lectures at IBDregistry.net

Charter Member of APIII,

Association for Pathology Informatics

Tim Vitale, “Film grain, Resolution, and Fundamental Film Particles”, Version 20, April 2007, available at: http://cool.conservation-us.org/coolaic/sg/emg/library/pdf/vitale/2007-04-vitale-filmgrain_resolution.pdf

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