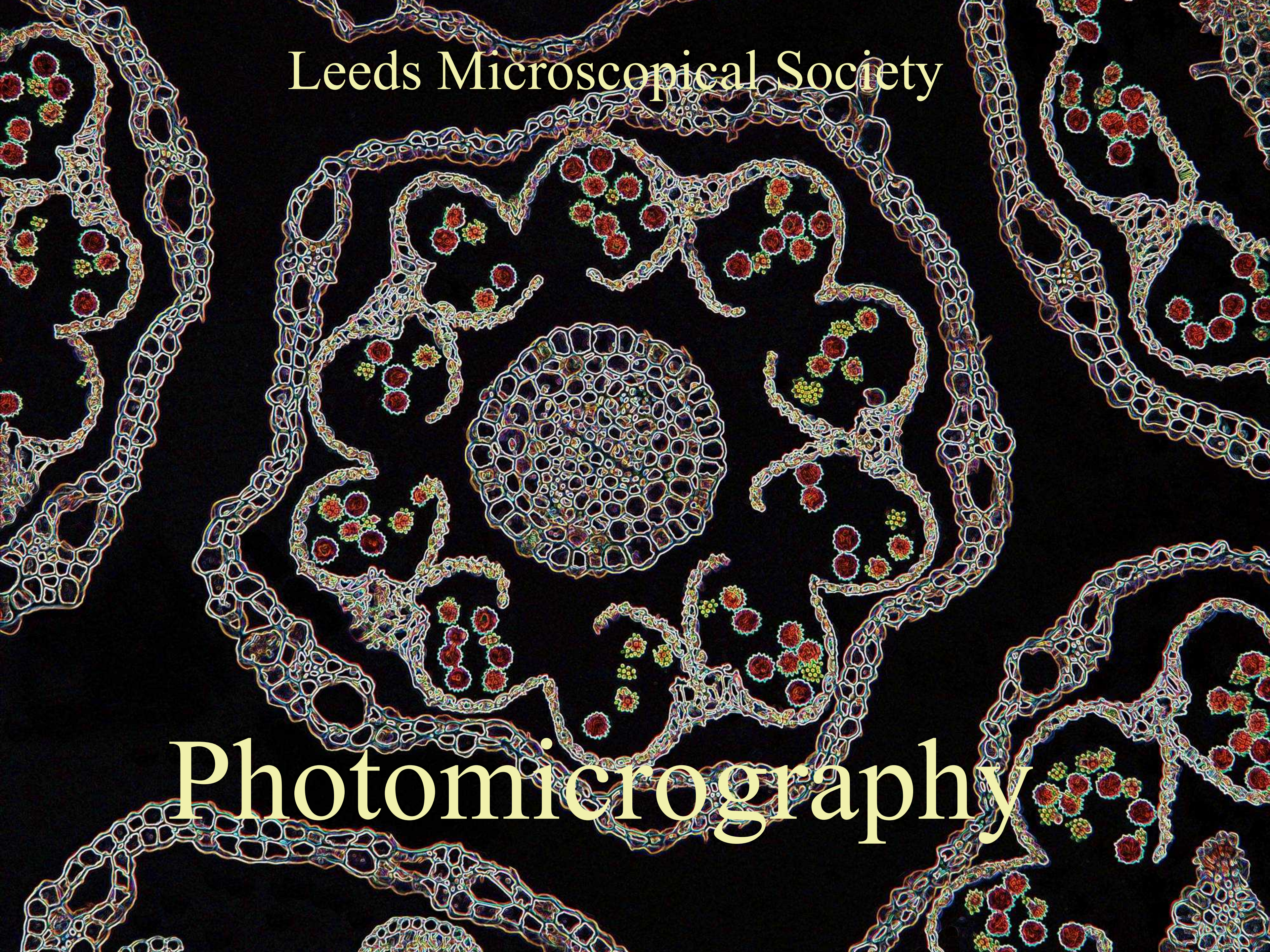
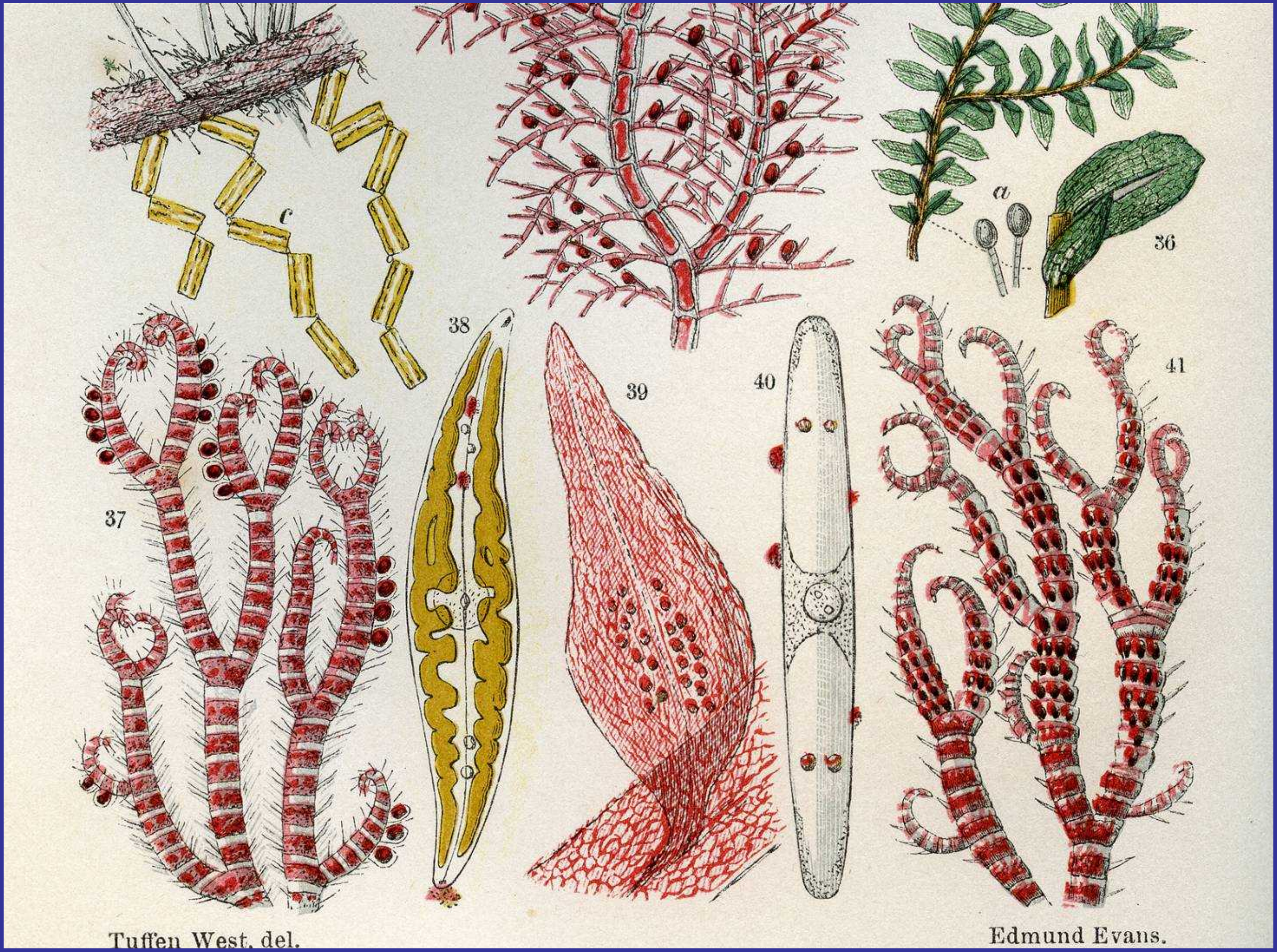
Leeds Microscopical Society

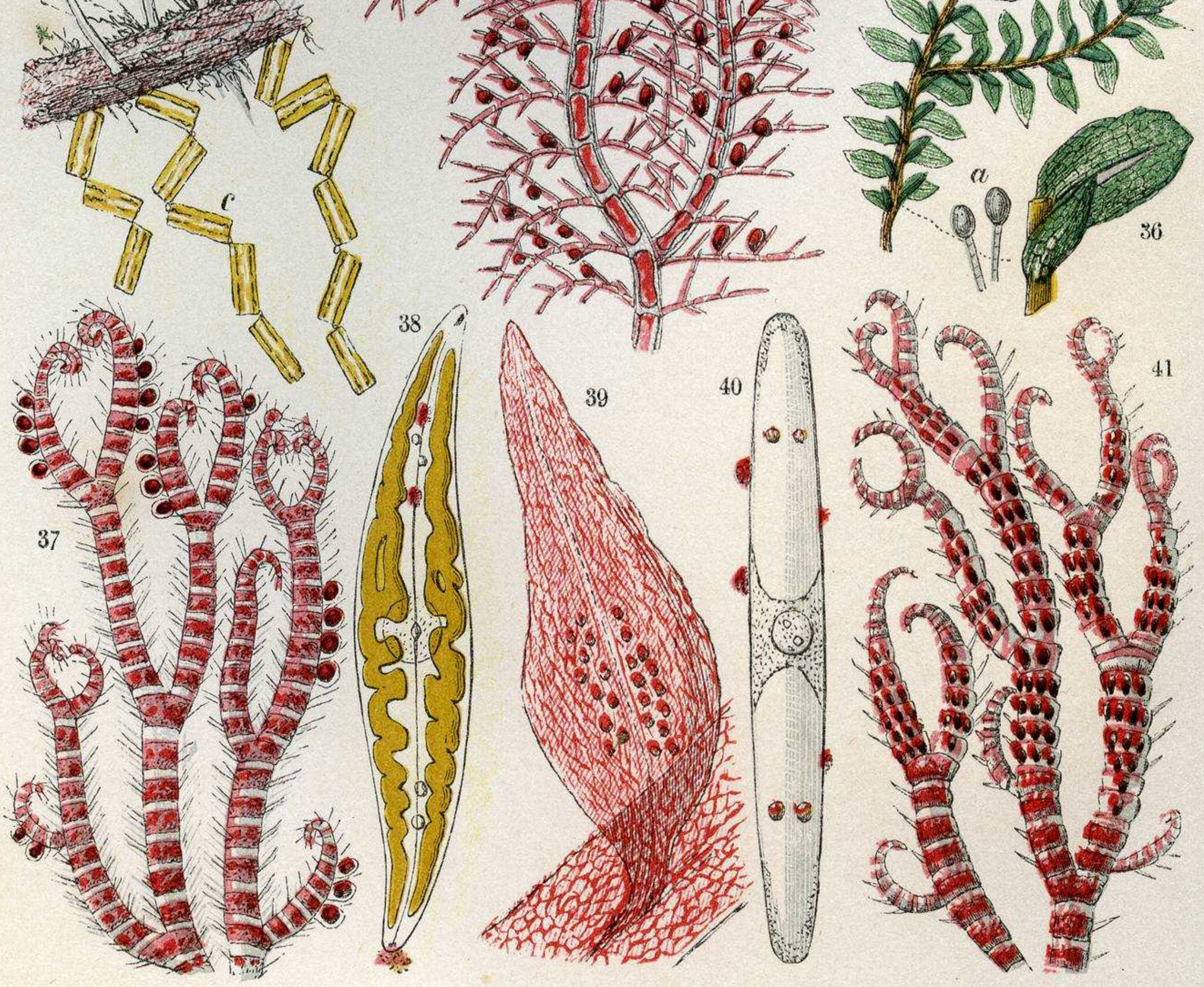


Photomicrography

Drawing- the first kind of "imaging".



Desmids, diatoms and algae from Hogg: History of the Microscope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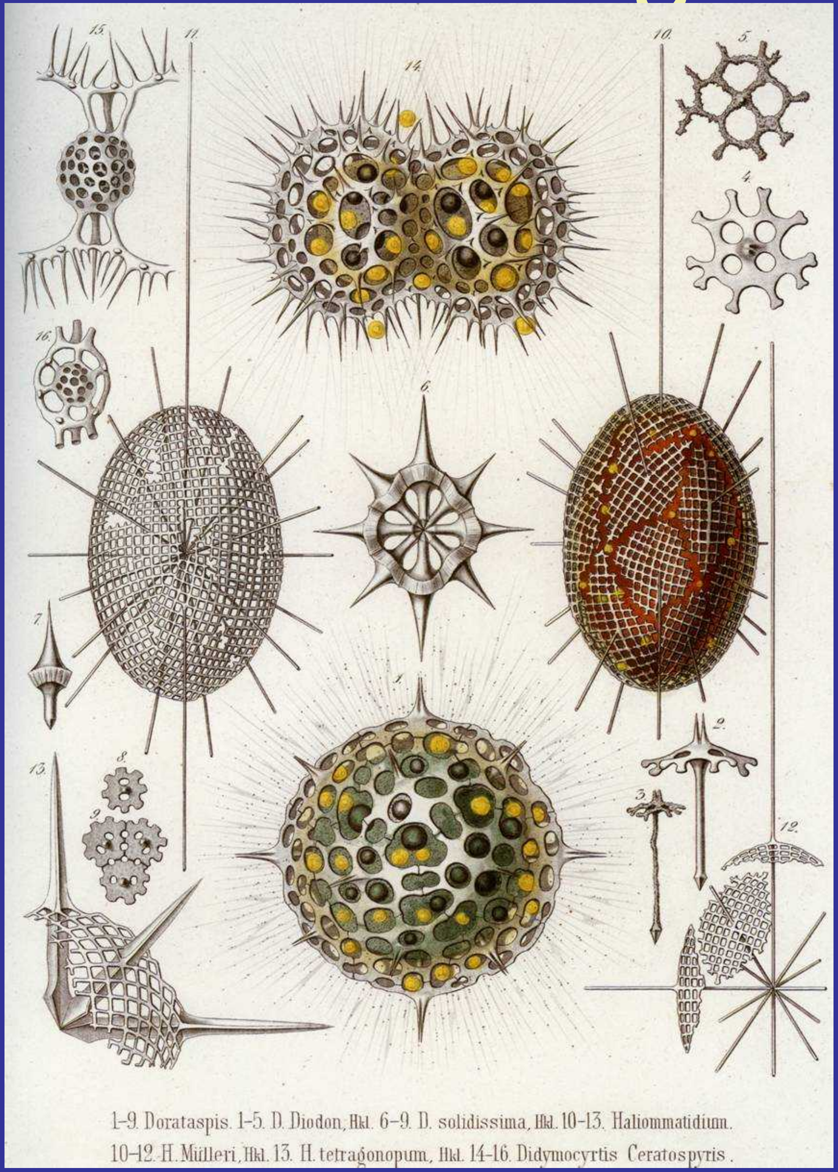
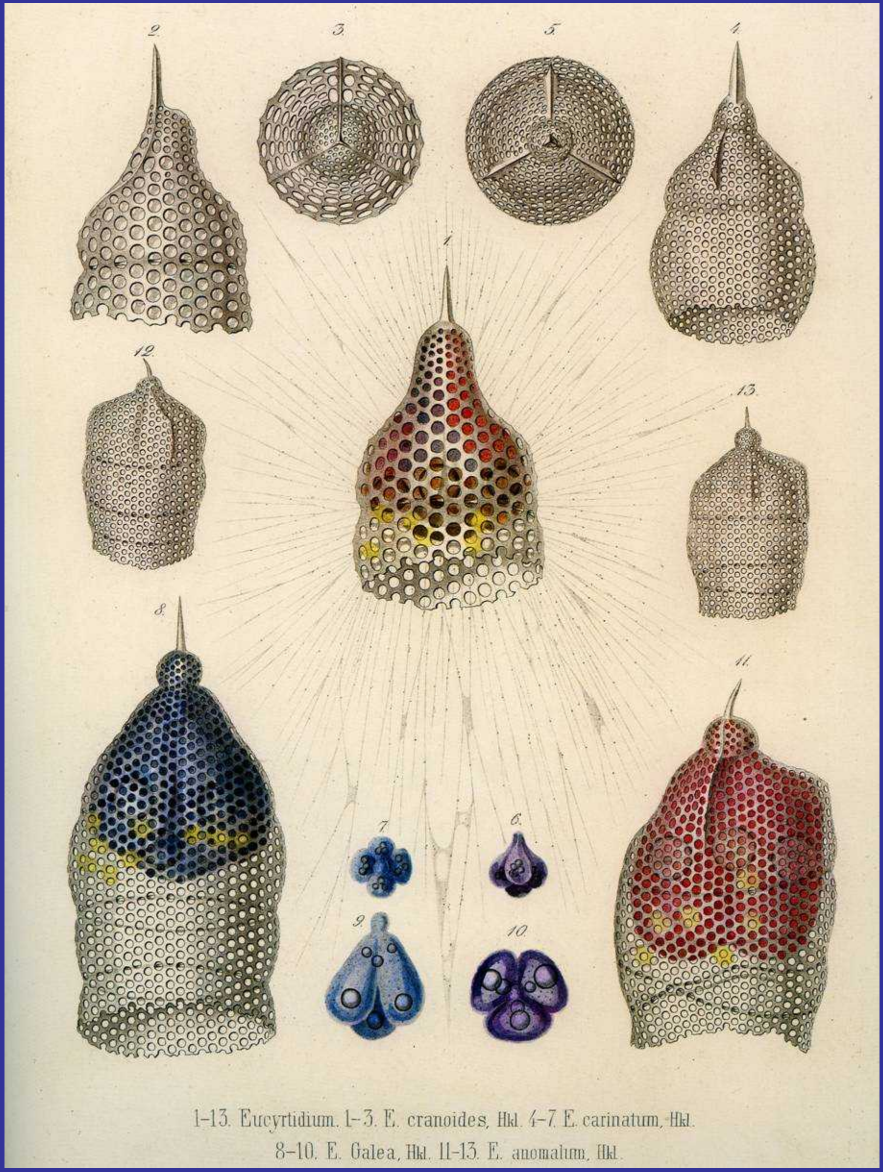
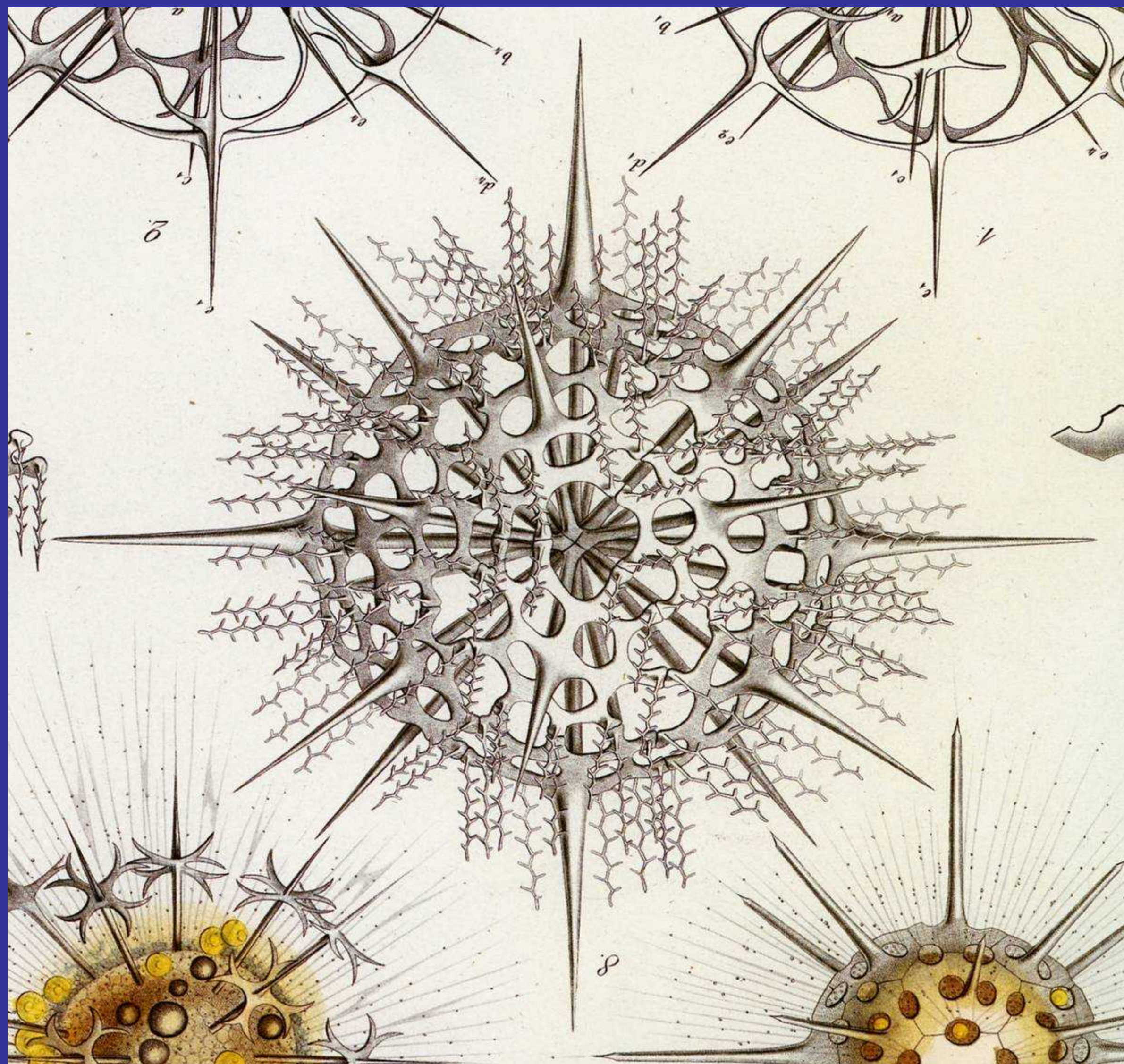
The drawings of Ernst Haeckel.





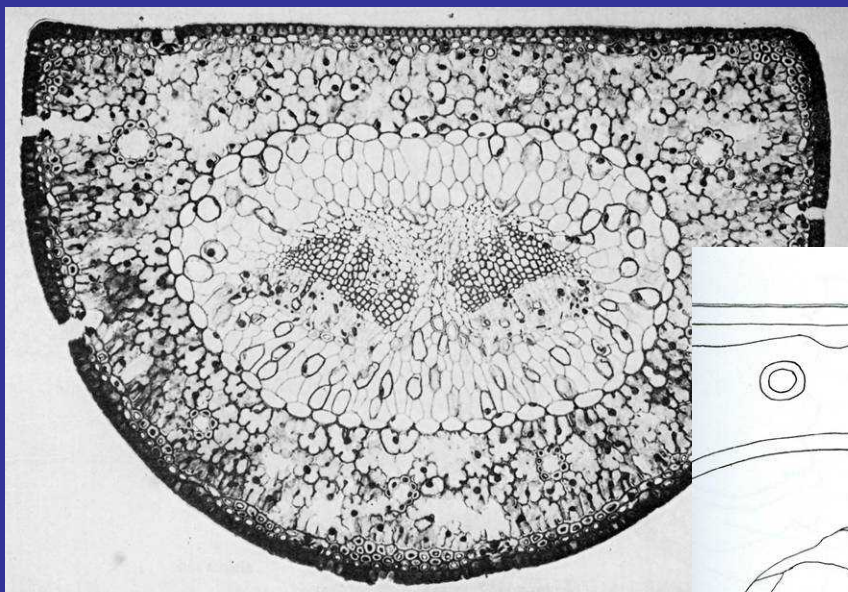
The drawings of Ernst Haekel.

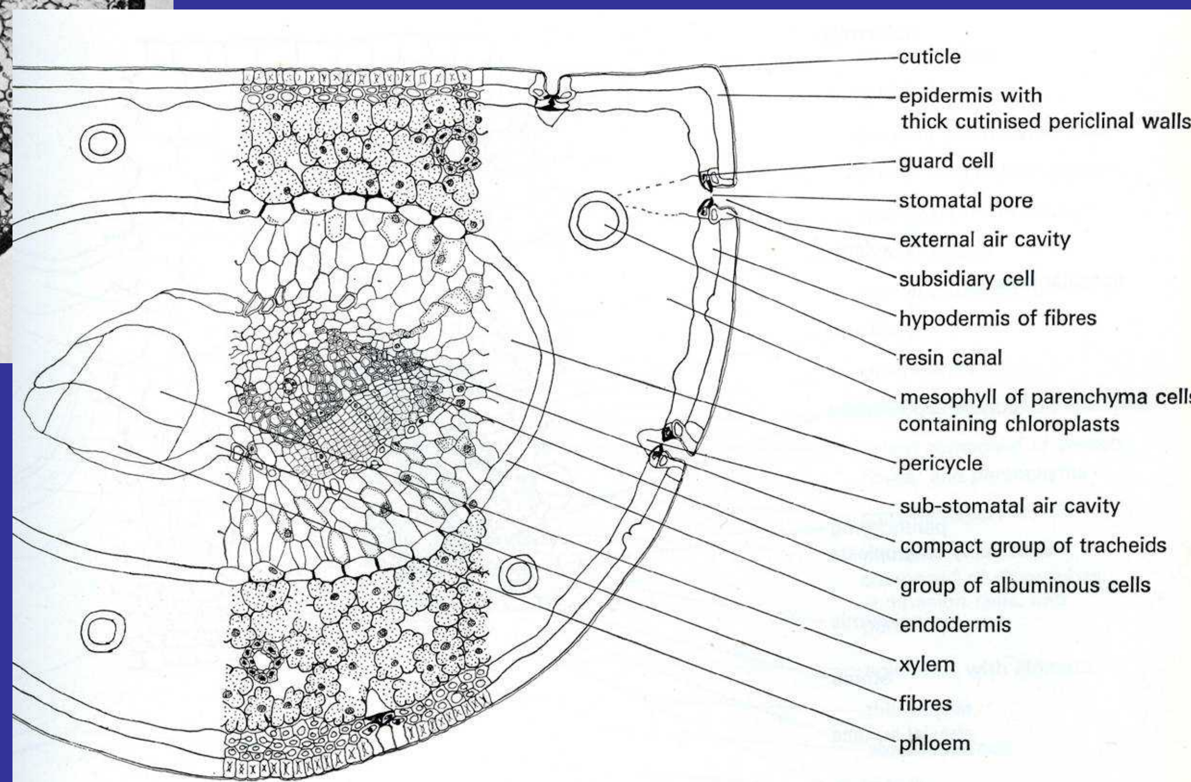


The drawings of Ernst Haeckel.

Haeckel's microscope

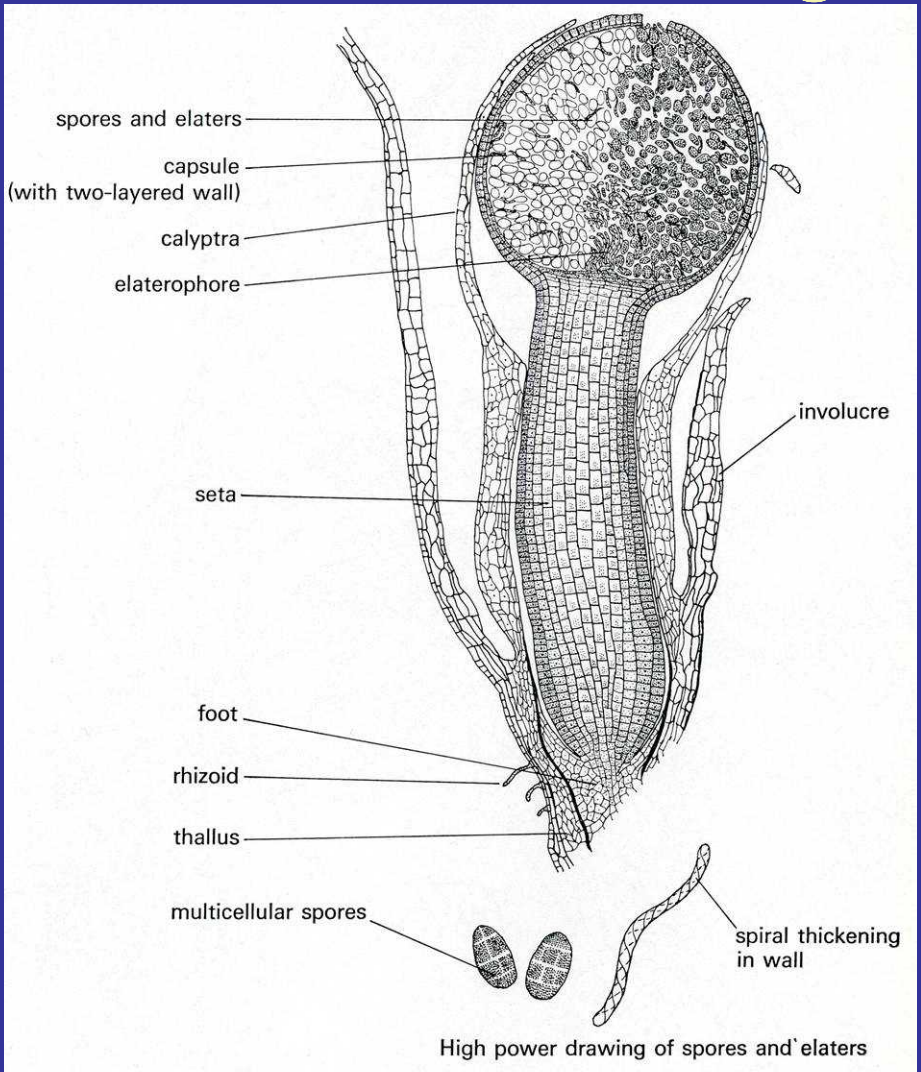
Drawings can still be more informative that a photographic image.





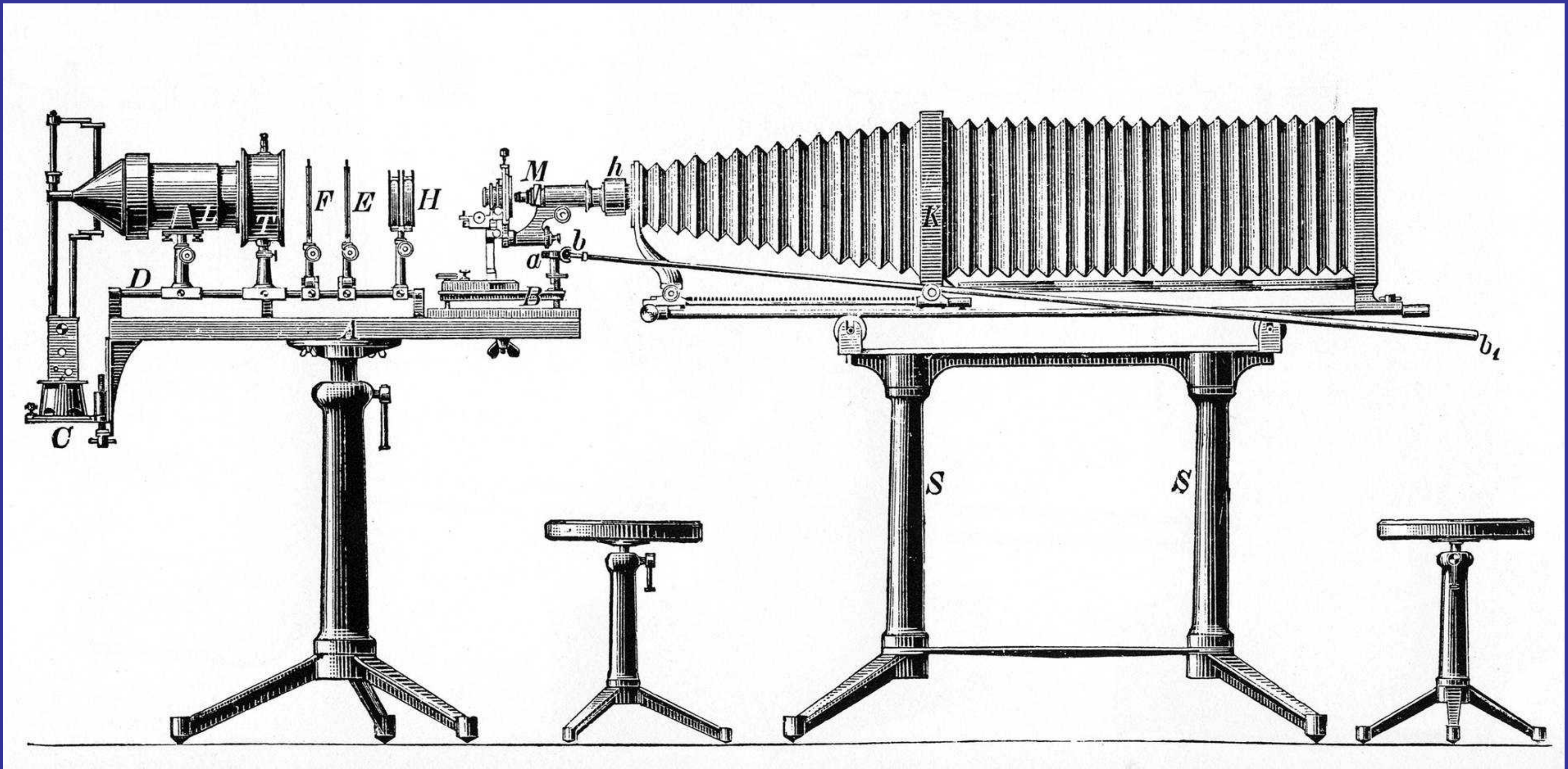
T.S. Scots pine leaf. Bracegirdle and Miles: An Atlas of Plant Structure.

Drawings can still be more informative that a photographic image.

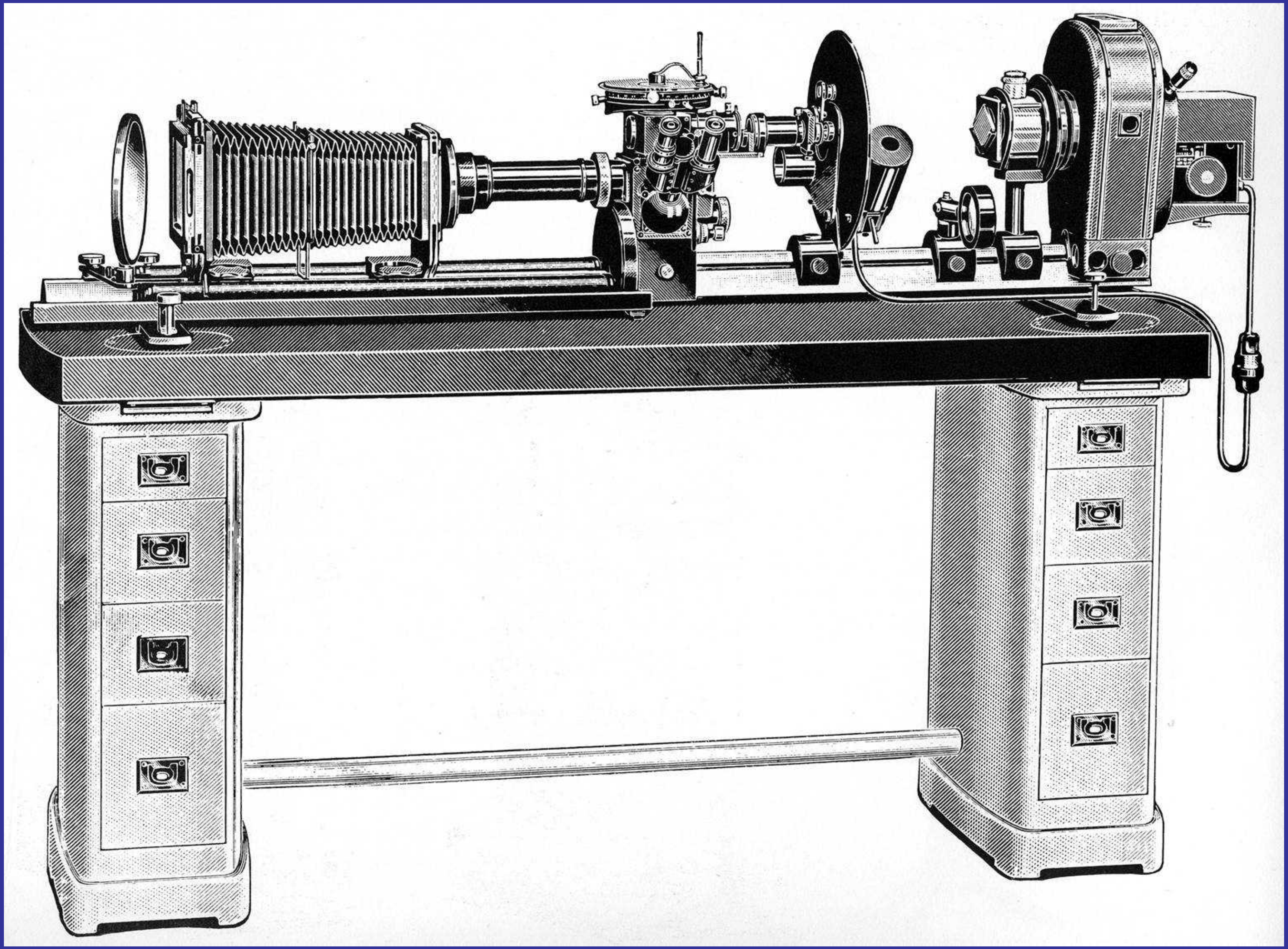


Pellia sporangium. Bracegirdle and Miles: An Atlas of Plant Structure.

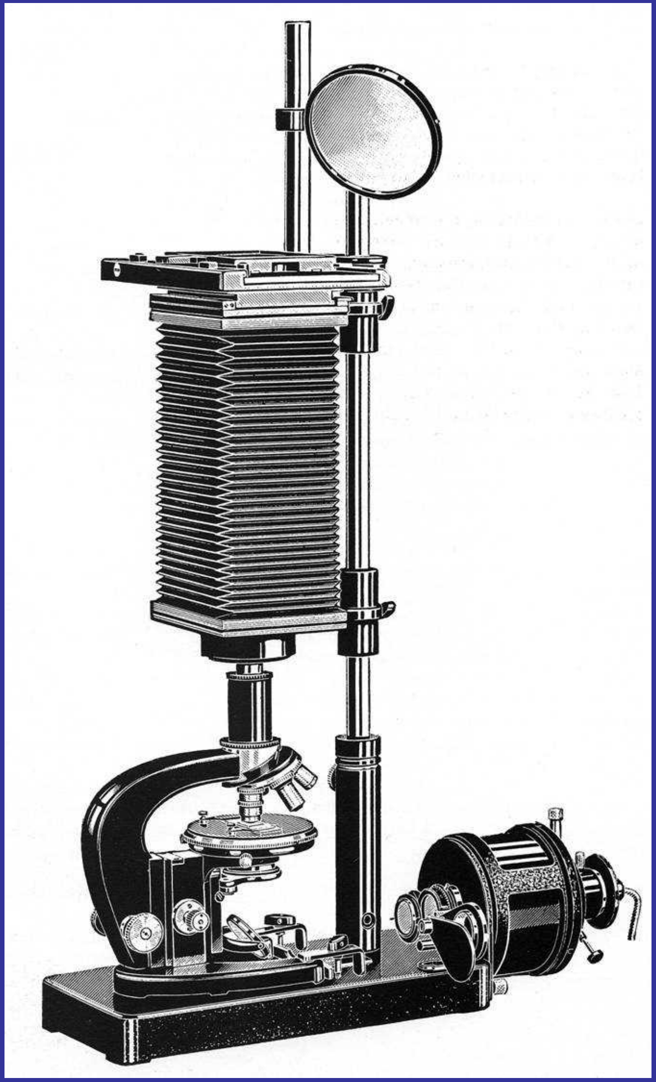
Photomicrography first using optical bench principles.



Photomicrography first using optical bench principles.



Photomicrography with attachment plate camera.



Zeiss stand L microscope with plate camera 1932.

Miniature plate cameras.

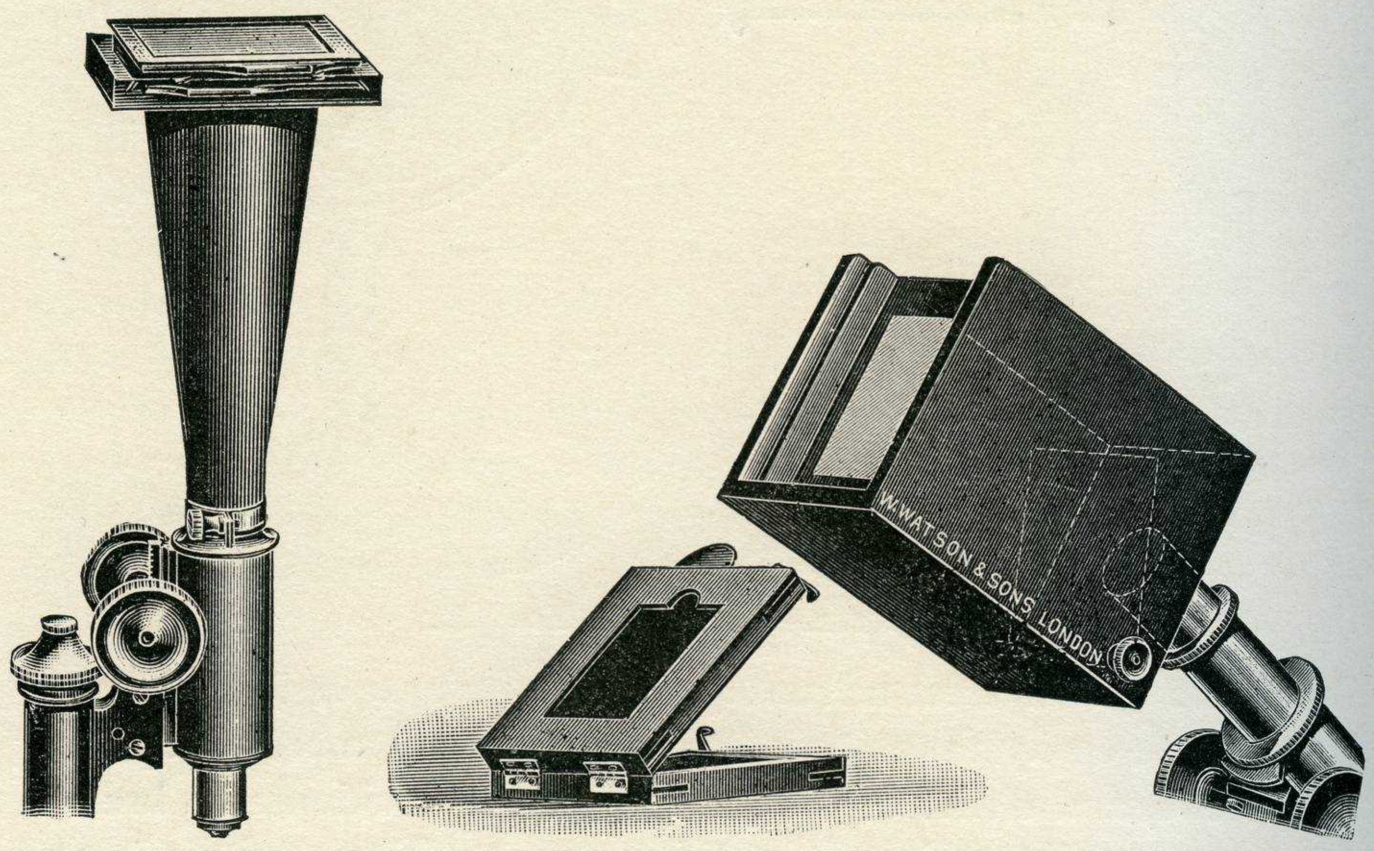
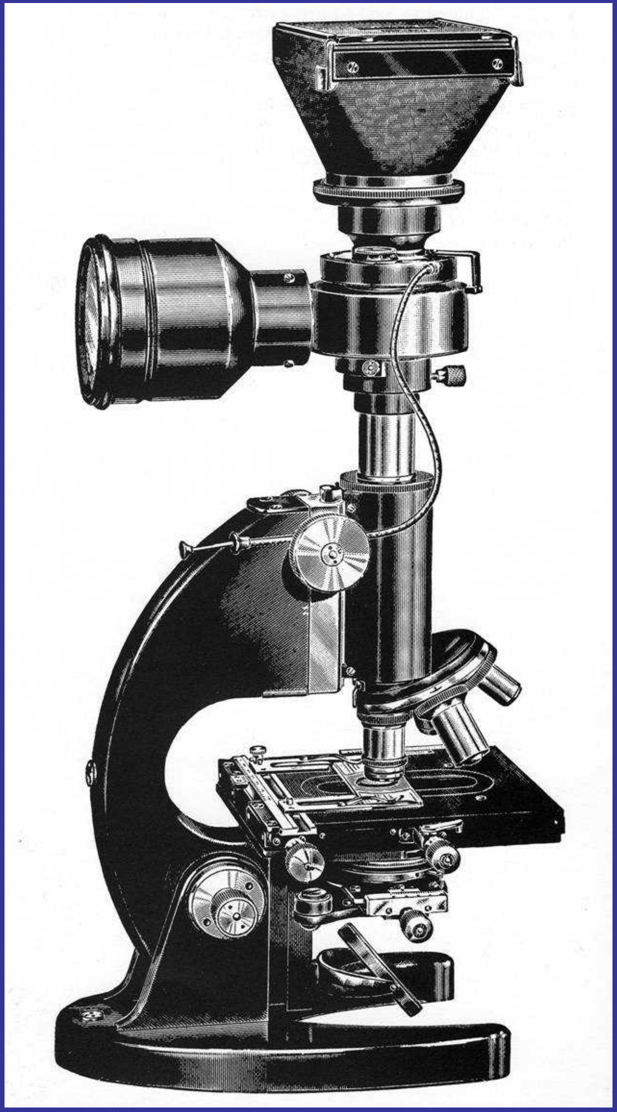


Fig. 223. Fig. 224.

Cameras for Fixing on the Microscope.

Spitta: Microscopy (1909), p444 of over 500 pages and is the single reference to photography.

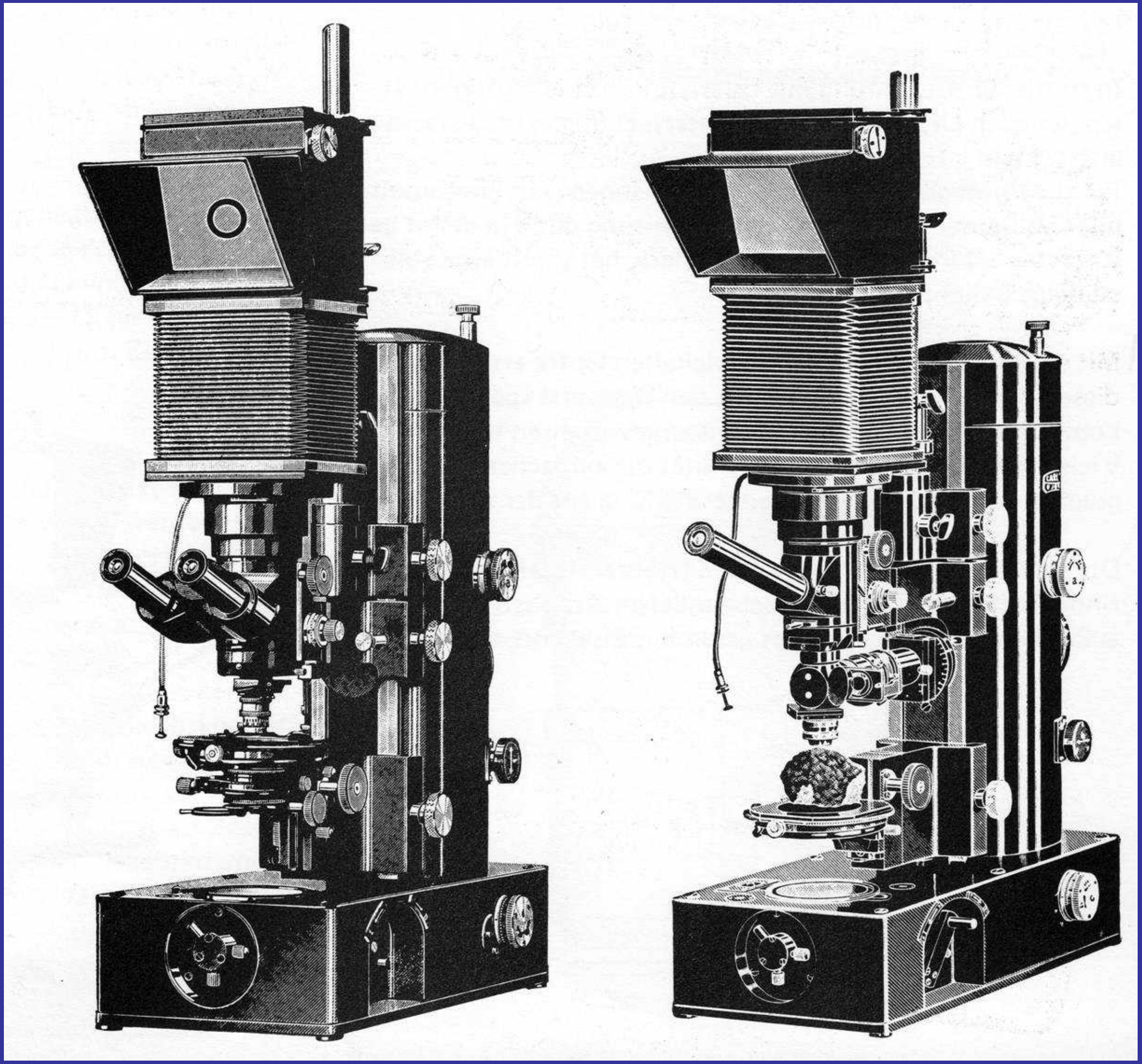
Miniature plate cameras.



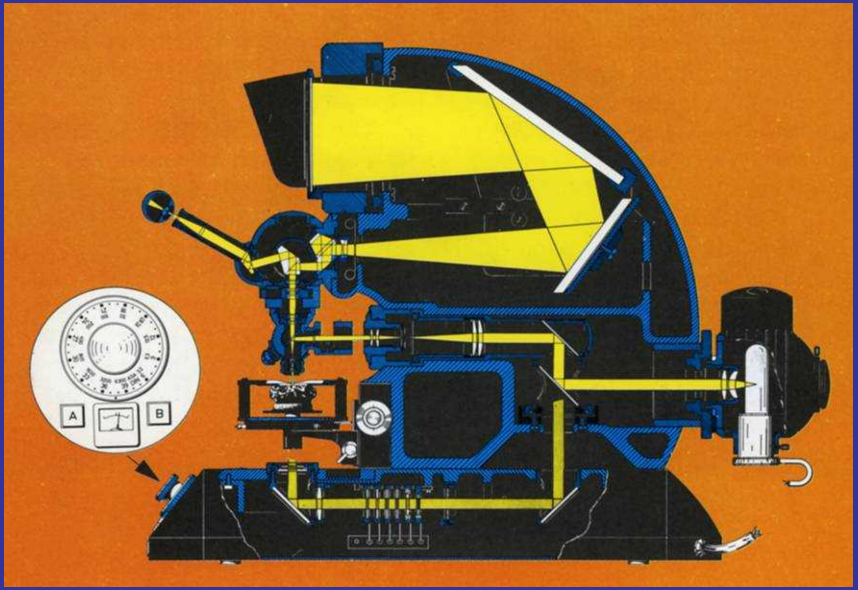
Pre-war 6x9 cm plate camera
and focussing viewfinder.

The first integrated cameras.

Zeiss Ultraphot in transmitted and reflected light configurations.

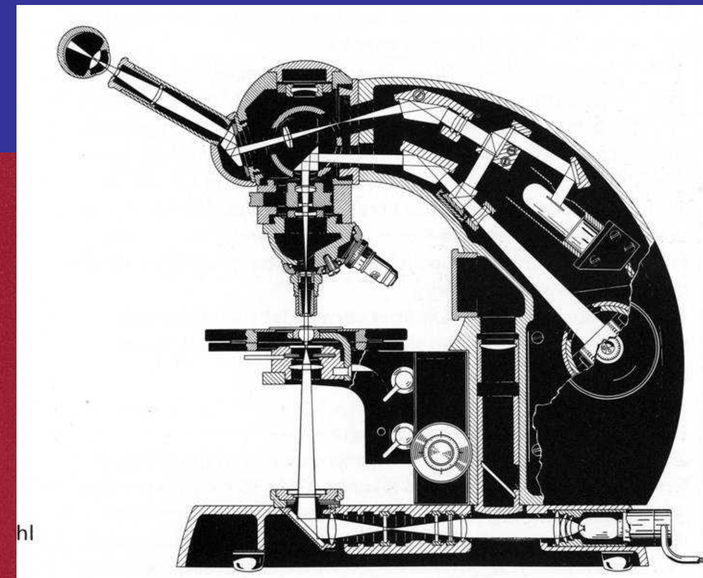


Universal photomicrography systems.



Zeiss Ultraphot II and III (seen here) could make 5”x4” and 35mm photographs in all known techniques.

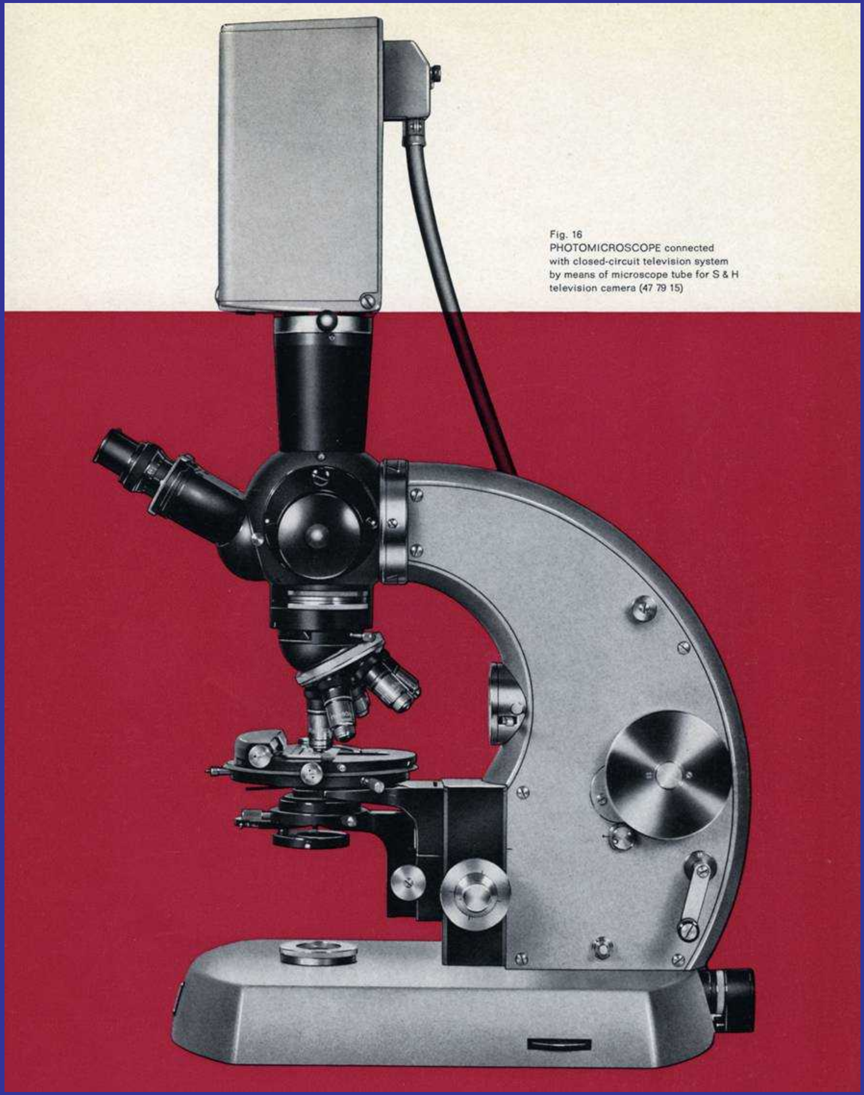
The first integrated 35mm system.



Zeiss Photomicroscope in 1966.

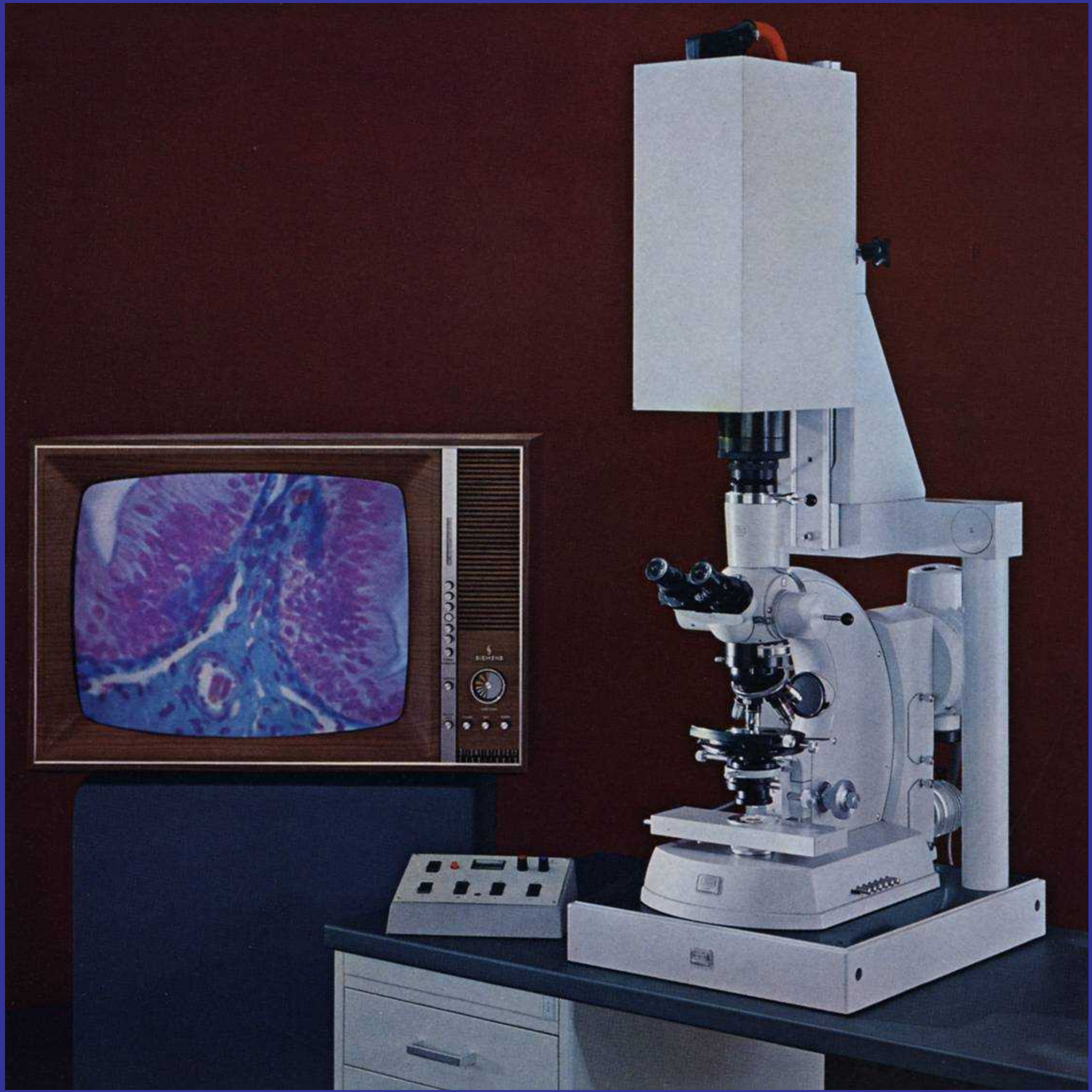
Early CCTV.

Monochrome CCTV camera on a Zeiss Photomicroscope in 1966.

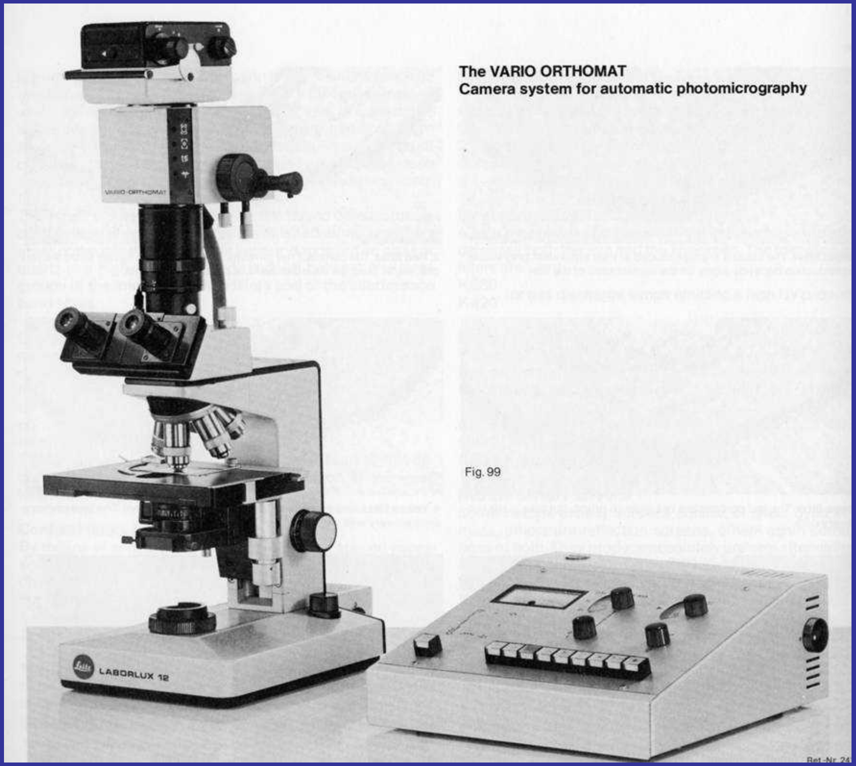


Early colour CCTV.

Zeiss Universal microscope with Siemens colour CCTV camera in 1970.



Attachment 35mm cameras.



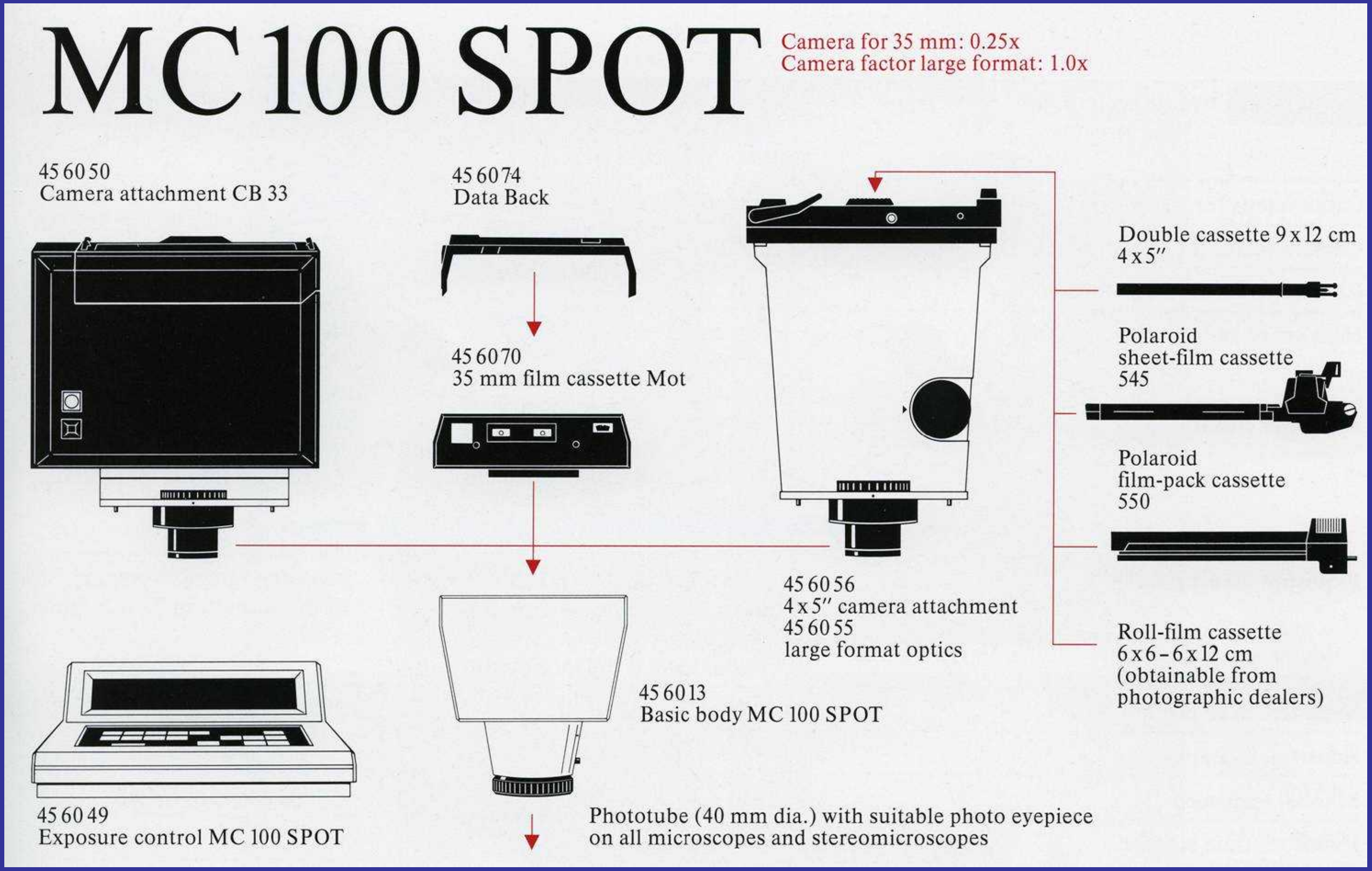
Attachment 35mm cameras.

Part of Zeiss MC 63 system with adapters for SLR cameras.

Attachment 35mm cameras.

Zeiss Axioplan microscope with MC 100 Spot system.

Attachment 35mm cameras.



Attachment 35mm cameras.

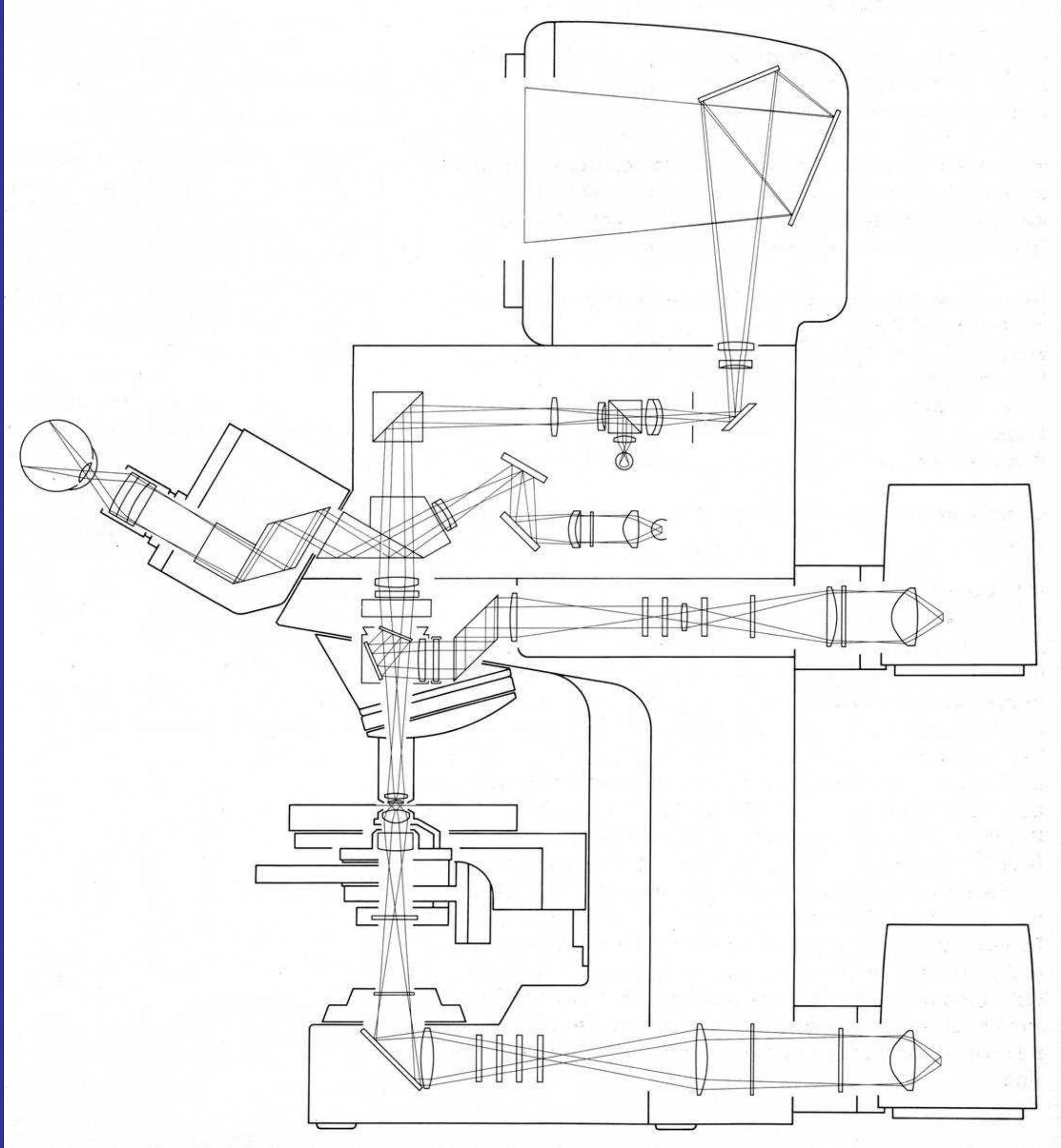
Zeiss MC 100 Spot camera body and film cassette with DX coding and selectable ISO film speeds.

Integrated photomicrography.

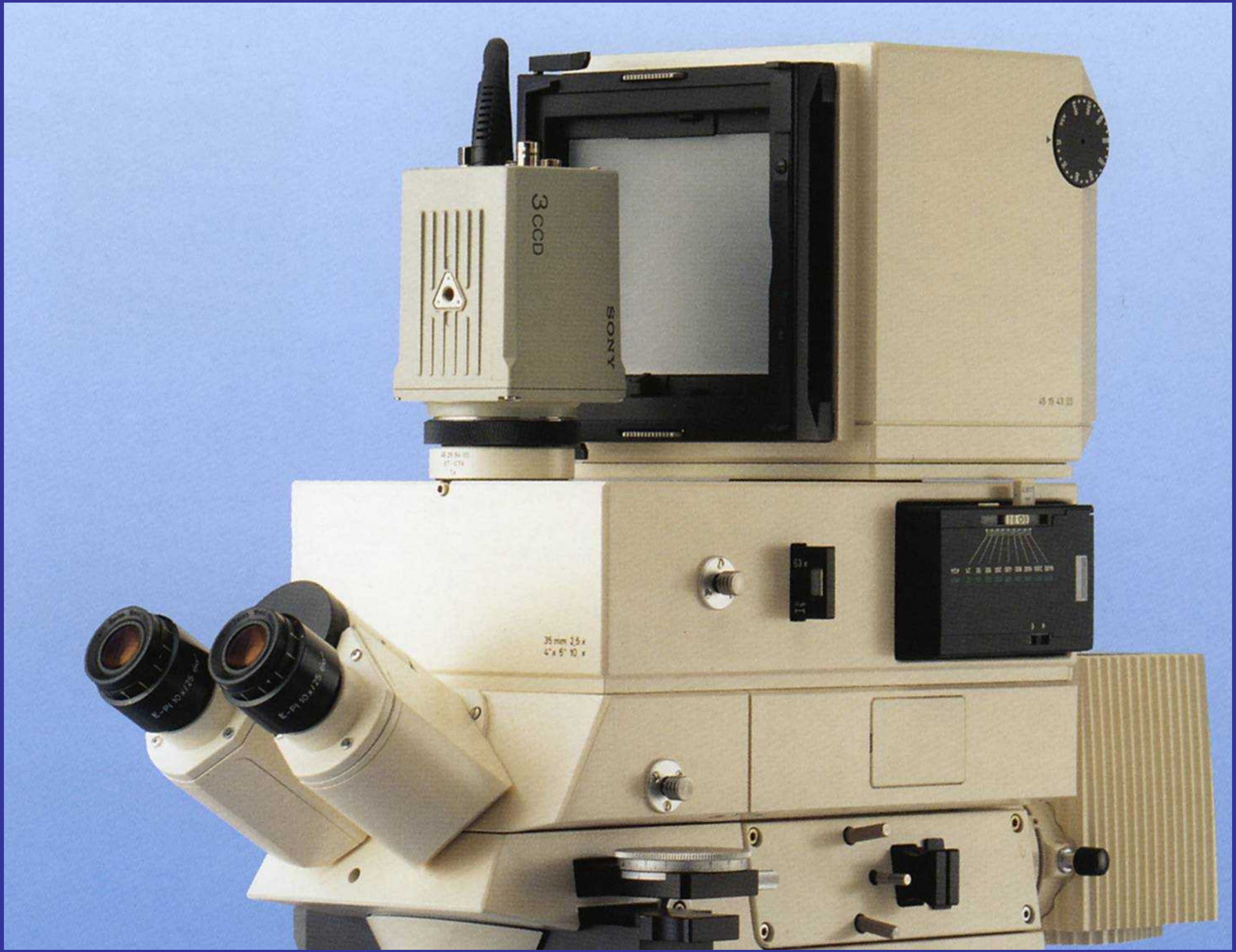
Zeiss Axiophot Photomicroscope with two 35mm cameras, 5”x4” camera and CCTV camera.

Integrated photomicrography.

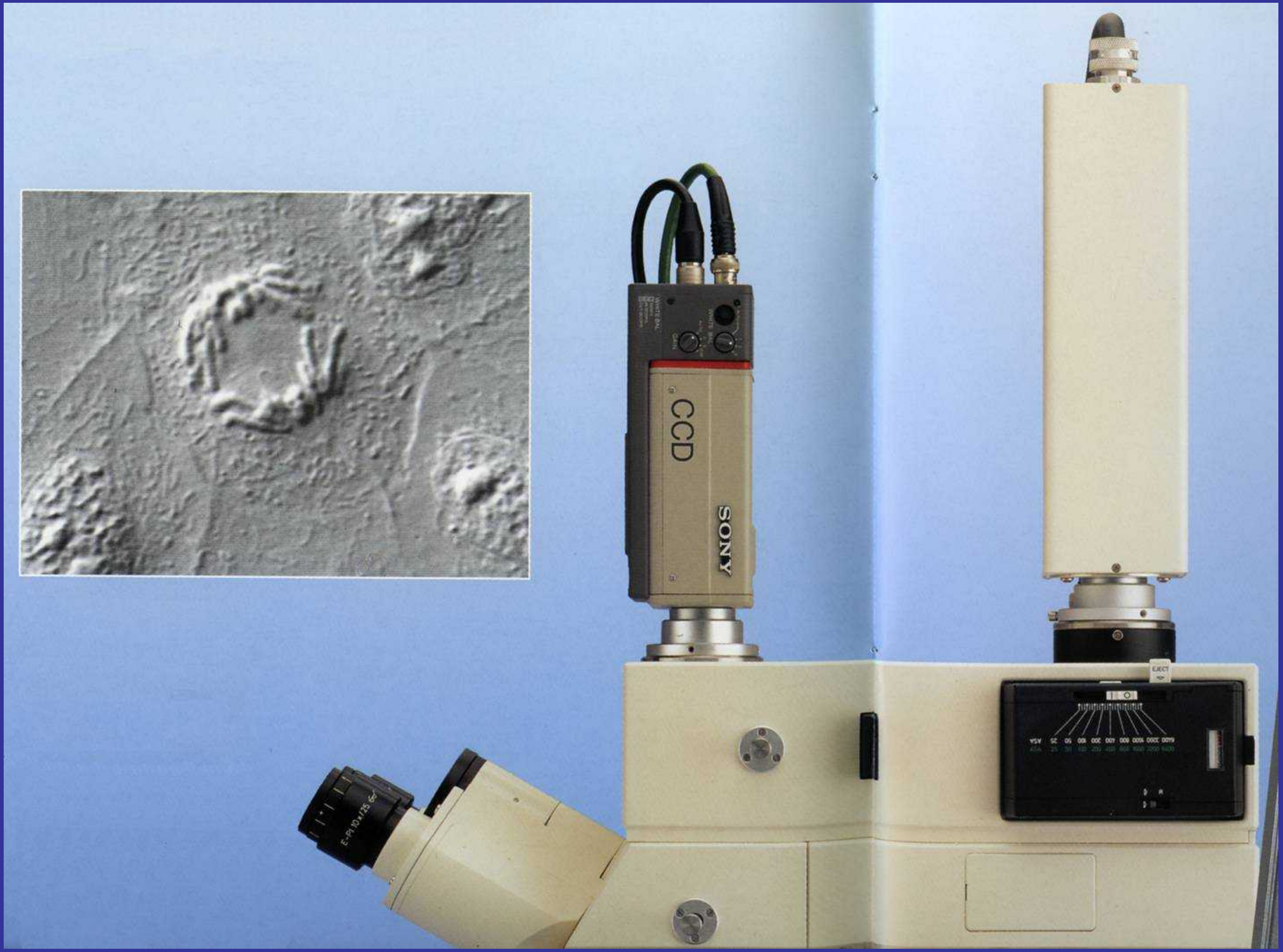
Zeiss Axiophot Photomicroscope with two 35mm cameras, 5”x4” camera and CCTV camera.



Integrated photomicrography.



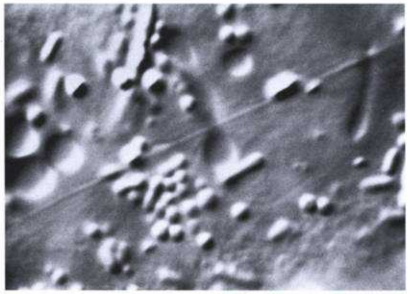
Similar system but CCTV based.

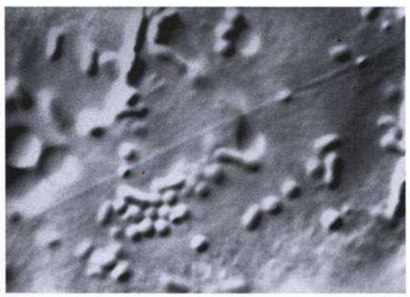


1988: Last developments of analogue technology.

Zeiss Analogue Contrast Enhancement system using 1” “Pasecon” 960 line tube TV camera.

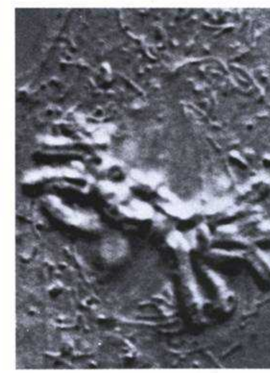
Last developments of analogue technology.





Direct visualization of cytoskeleton-transport fibrils with associated vesicles and neighbouring cisternae of the endoplasmic reticulum in the cytoplasm of living plant epithelium cells (allium cepa). The structures are shown in detail on page 12; the sequence on the left shows their dynamics and changes in shape (time interval 10 s).

Transport of mitochondria and vesicles along cytoskeletal structures between the perinuclear (Golgi) area and the cell periphery in a PtK_2- cell.



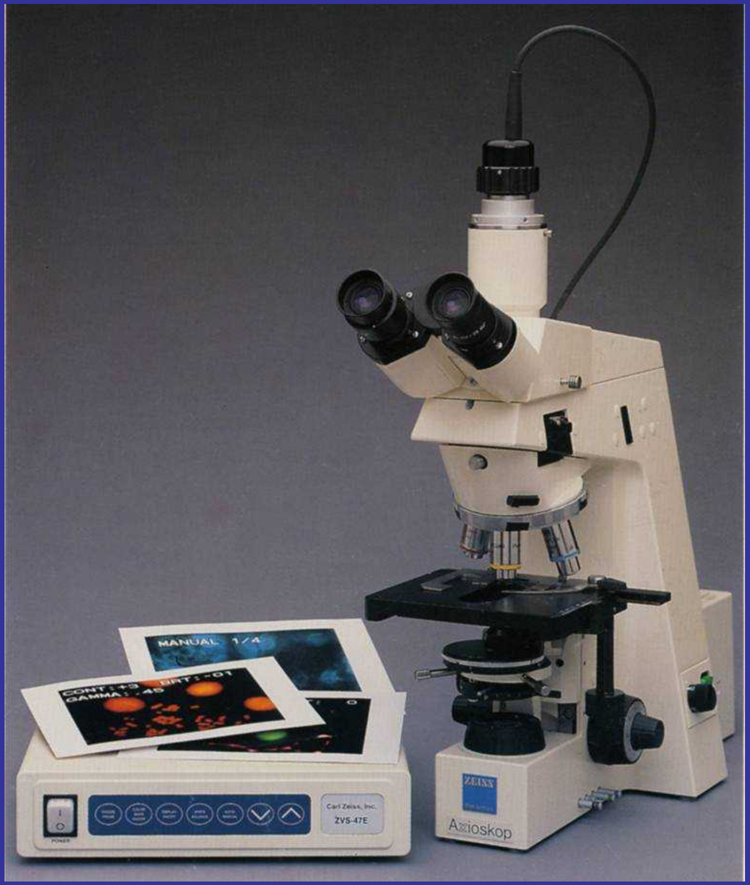
PtK_2 cells in mitosis
Top: ultra-fine cell processes in contact with neighbouring cells.
Bottom: chromosomes in metaphase.

All photographs taken with ACE Microscope System with universal *Axioplan* Microscope, *Plan-Neofluar* objective 100x/1.3 Oil Pol; condensor front lens 1.4 Pol; DIC; additional magnification 4x; final magnification on the ACE monitor 8800:1.

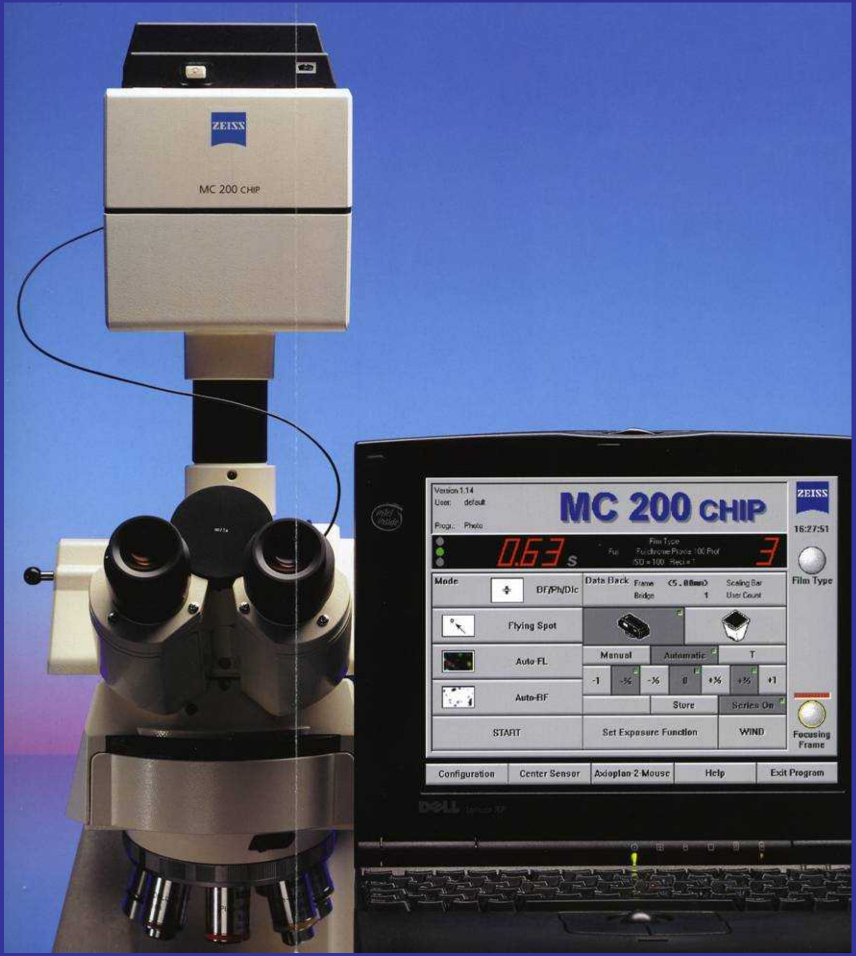
N RESEARCH

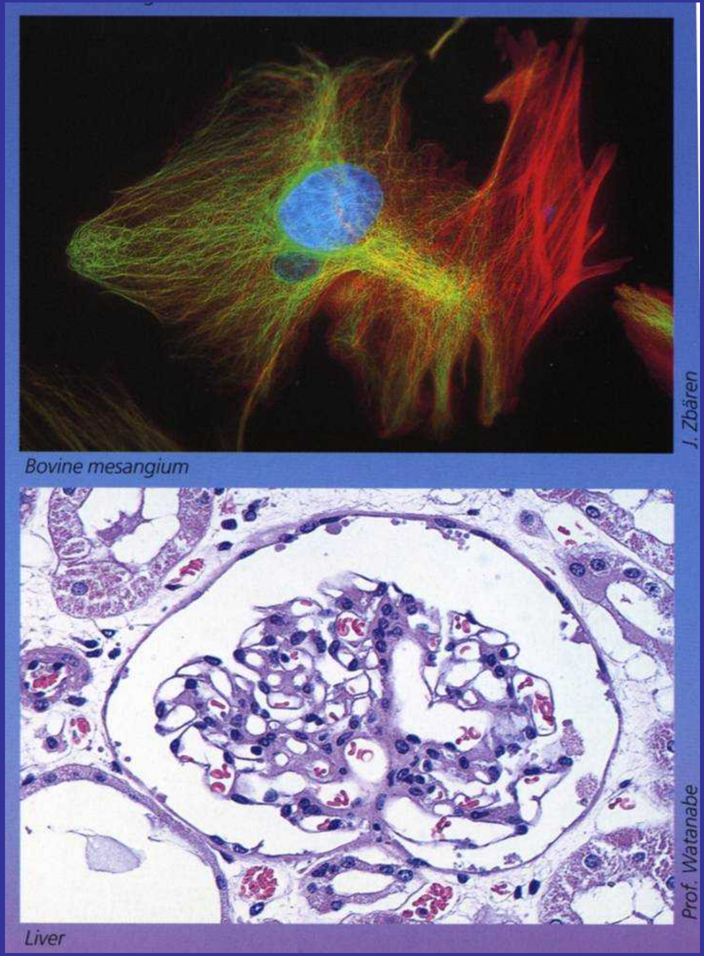
Last developments of analogue technology.

Several types of video printing direct from CCTV camera and monitor: thermal, dye sublimation and laser printing.



1996: Last developments of film technology.





Zeiss MC 200 Chip with CCD sensor to measure tonal range across the image.

The development of digital imaging.

- Monochrome CCTV camera Sony XC-77.
- Made to integrate for imaging dim objects.
- Frame grabber in computer to acquire successive images taken in fluorescence.
- False colours assigned in computer and overlaid.

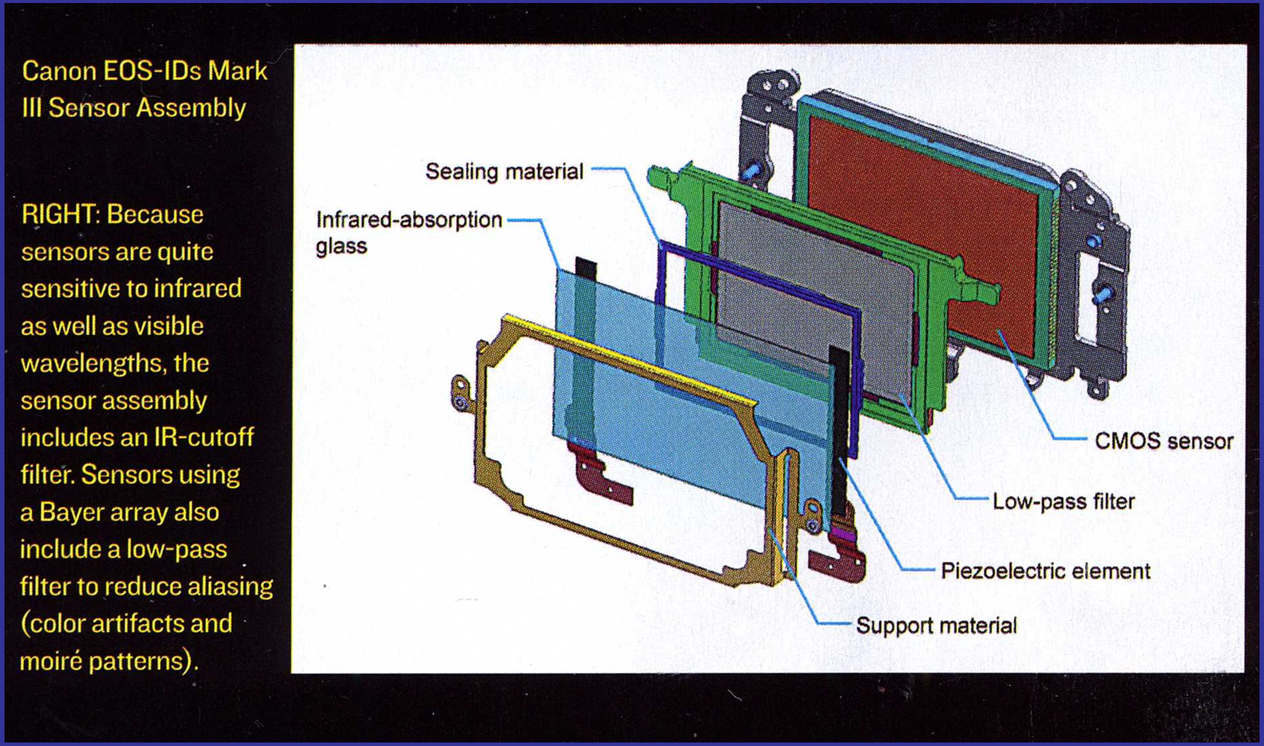
The development of digital imaging.

- Early digital still cameras not supported by microscope manufacturers.
- Poor image quality.
- Poor integration with computer.

Then, suddenly:

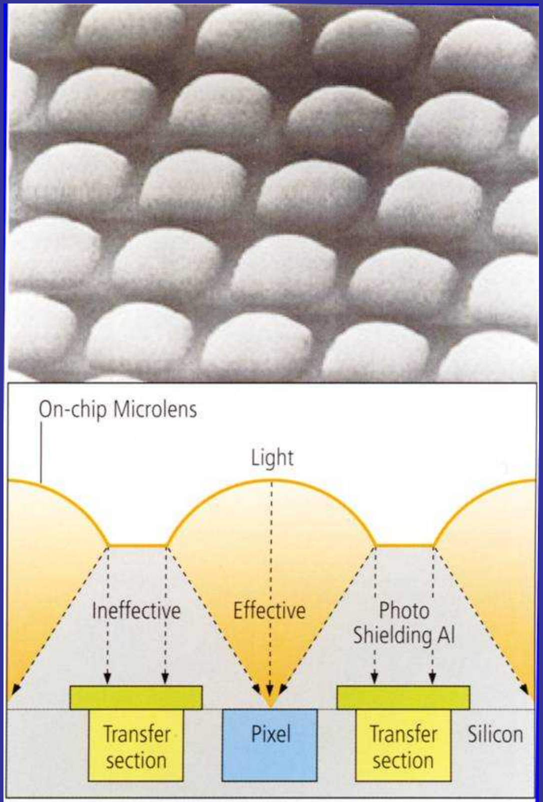
Major microscope manufacturers launched dedicated digital cameras.

Digital sensors.



Many layers comprise a digital sensor but the active layer consists of an array of diodes.

Digital sensors.



Maximum light efficiency of the AxioCam sensor through the microlens design.

The light-sensitive layer of the sensor contains an array of diodes. A diode responds proportionally to light by producing an electric current.

Digital sensors.

Two major types of digital sensor:

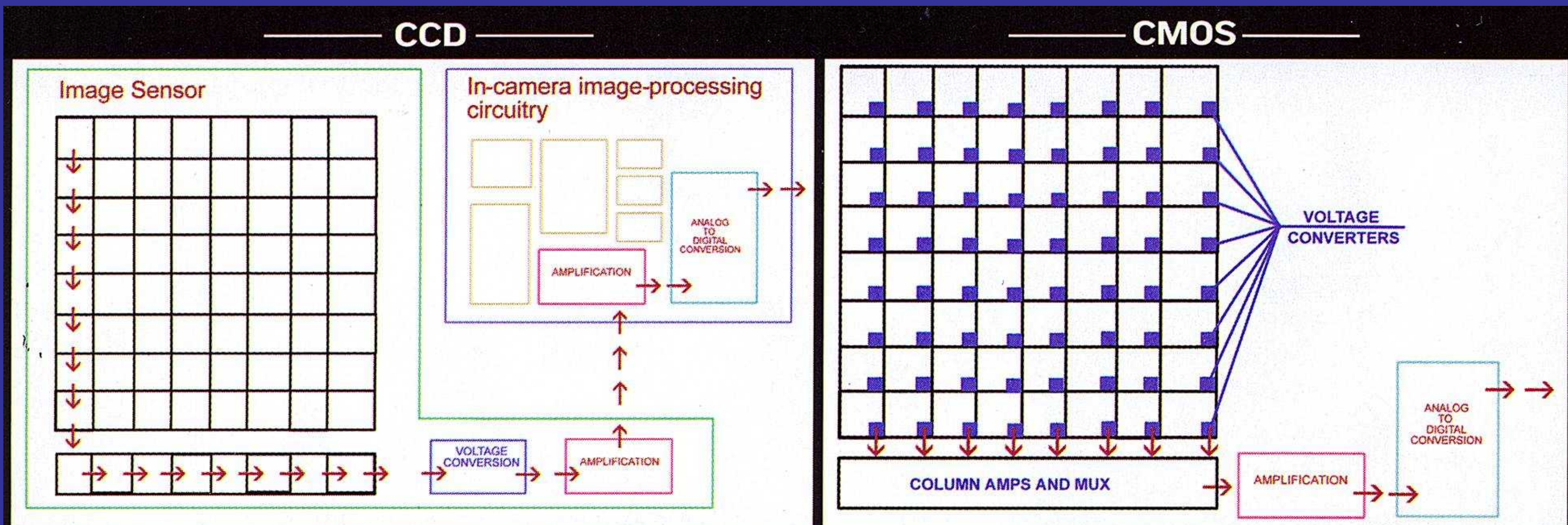
CCD (Charge Coupled Device)

CMOS (Complementary Metal Oxide Sensor)

In both cases, the sensor comprises an array of diodes.

Each diode becomes a picture element = **PIXEL**.

Digital sensors.



ABOVE, LEFT: In a CCD sensor, charges created when light photons strike the photosites are transferred to the edge of the chip where they're converted to voltage, one row at a time. They're amplified off-chip and sent to the A/D converter to be converted to digital form. This method is accurate but slow and involves a lot of off-chip circuitry. ABOVE, RIGHT: Each pixel in a CMOS sensor has its own amplifier and voltage converter, so conversion to voltage is done at the pixel. It requires much less power to transfer voltage than to transfer current, so CMOS sensors provide longer camera-battery life. Also, multiple channel reading speeds up operation, and on-chip noise reduction is possible.

Digital sensors.

For microscopy the CCD sensor has always been the better performer due to low noise with dim objects (especially fluorescence) and long exposure.

CMOS sensors are almost universally used in consumer cameras from inexpensive compacts to professional SLR cameras. They have improved enough to be good also for microscope cameras for non-fluorescence imaging.

Digital sensors.

A digital sensor is an analogue device!

After the electronic signals have left the sensor they enter an “analogue to digital converter”, before being sent to the computer via firewire or USB cable.

Digital sensors.

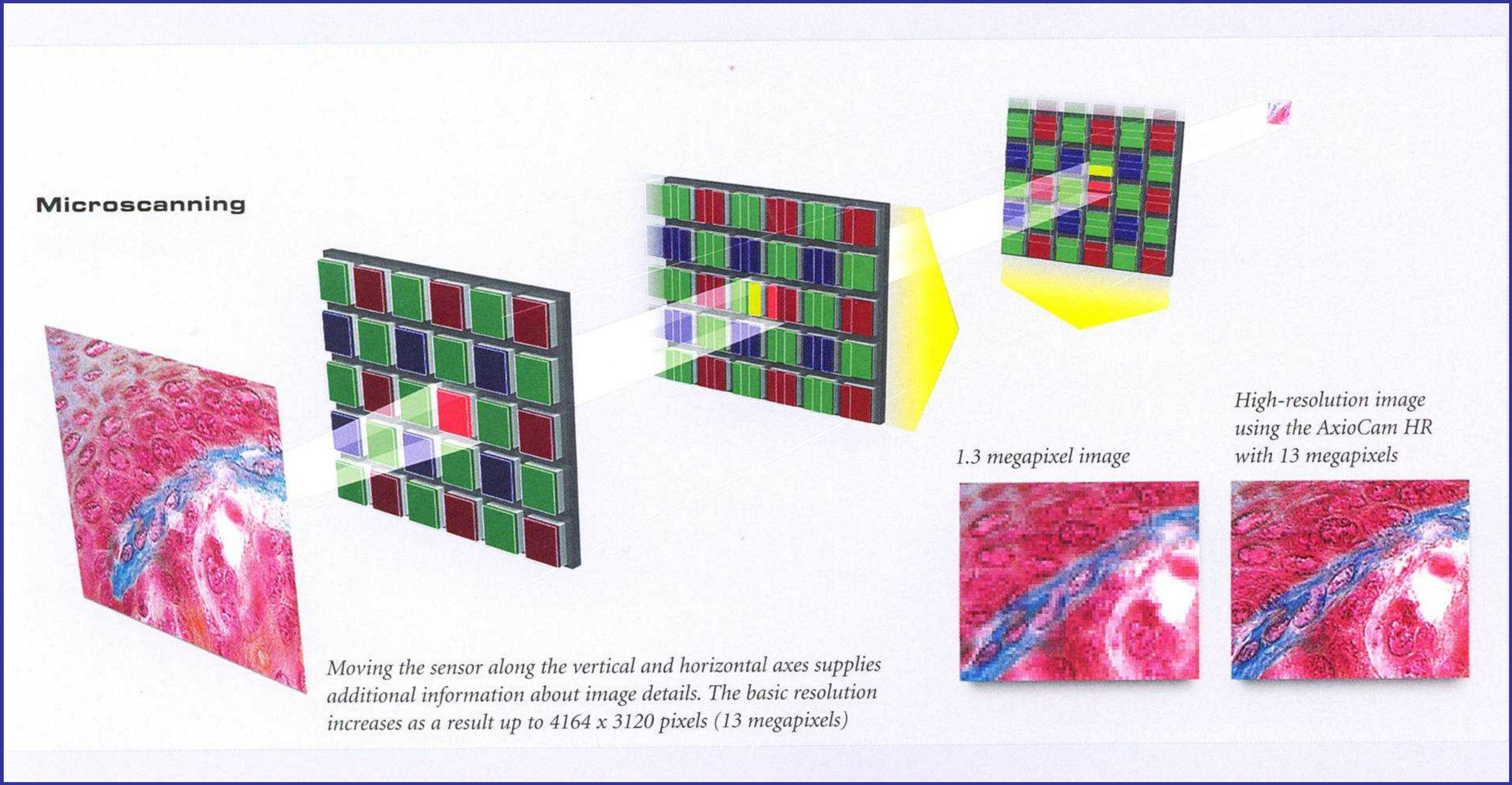
A digital sensor is a monochrome device!

Monochrome cameras can be used for colour imaging by taking three images through red, green and blue filters.

Colour cameras have a Bayer mask fitted in front of the pixel array. This reduces the light reaching the sensor.

Digital sensors.

Bayer mask



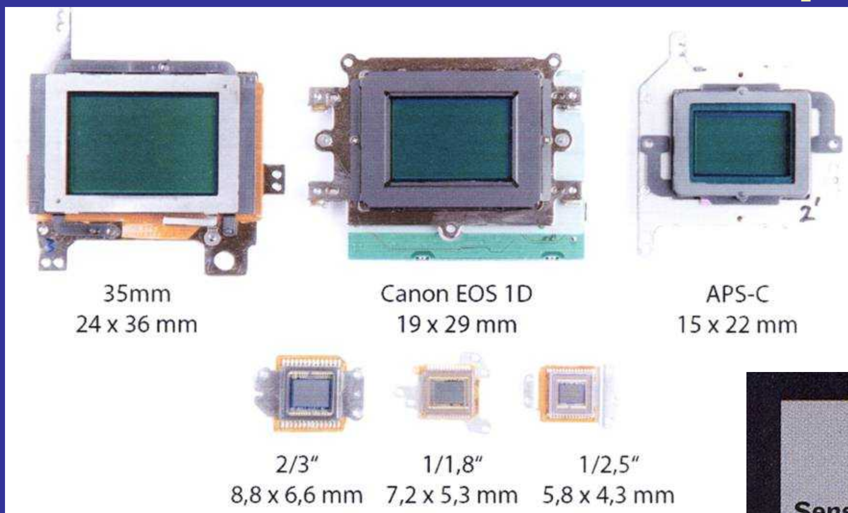
Digital camera types.

- Compact cameras with non-detachable lenses.
- Compact cameras with detachable lenses.
- Single Lens Reflex cameras.
- Digital camcorders.
- Mobile phone-type devices.
- Dedicated microscope cameras or astro cameras.

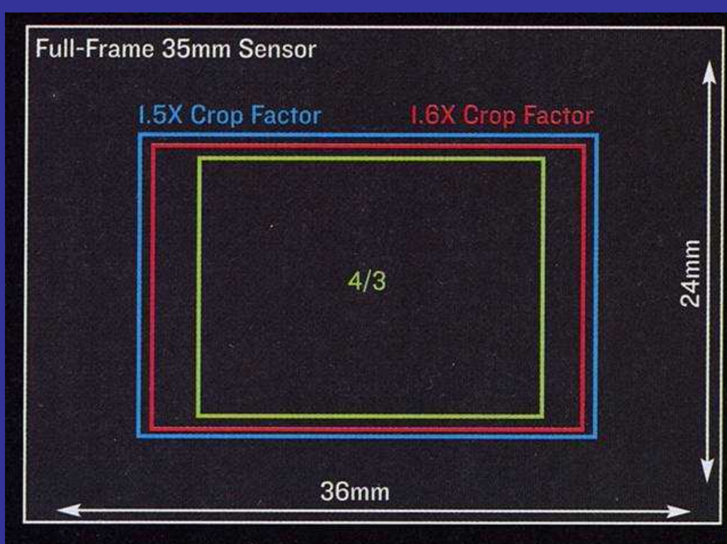
Factors affecting digital image quality.

- Numbers of pixels.
- Size of pixels.
- Dimensions of sensor.
- Quality of A-D conversion.
- Quality of image processing.

Factors affecting digital image quality.



Sensor size



D-SLR SENSOR SIZES

Sensor	Size	Magnification Factor	Camera Models
Full-Frame	36x24mm	1.0x	Canon EOS-1Ds Mark III Canon EOS 5D Nikon D3
APS-H	28.1x18.7mm 27.0x18.0mm	1.3x 1.33x	Canon EOS-1D Mark III Leica M8*
APS-C	23.6x15.8mm	1.5x	All Nikon D-SLRs except D3 All Pentax D-SLRs All Samsung D-SLRs All Sony D-SLRs
	23.0x15.5mm 22.2x14.8mm 20.7x13.8mm	1.5x 1.6x 1.7x	Fujifilm FinePix S5 Pro, IS Pro Canon EOS Rebel models, EOS 40D Sigma SD14
Four Thirds	17.3x13.0mm	2.0x	Leica Digilux 3 All Olympus D-SLRs All Panasonic D-SLRs

*A rangefinder model, not a D-SLR, but included because it's a serious interchangeable-lens camera

Factors affecting digital image quality.

- Number of pixels. It seems logical that more pixels provide more resolution of detail.
- This is only true up to a point.
- Smaller pixels collect less light, so to provide the same output their signals must be amplified more strongly.
- Amplification = heat = noise = grainy image.
- A 1 micrometer pixel is the smallest useful.

Factors affecting digital image quality.

- Therefore:

- Best image is from a large sensor with large pixels. For microscopy, 7mu or larger is optimal.
- E.g., Canon EOS 5D with 12 megapixels of 8.2 mu.
- Majority of serious pro microscope cameras have only 1.4 megapixels.

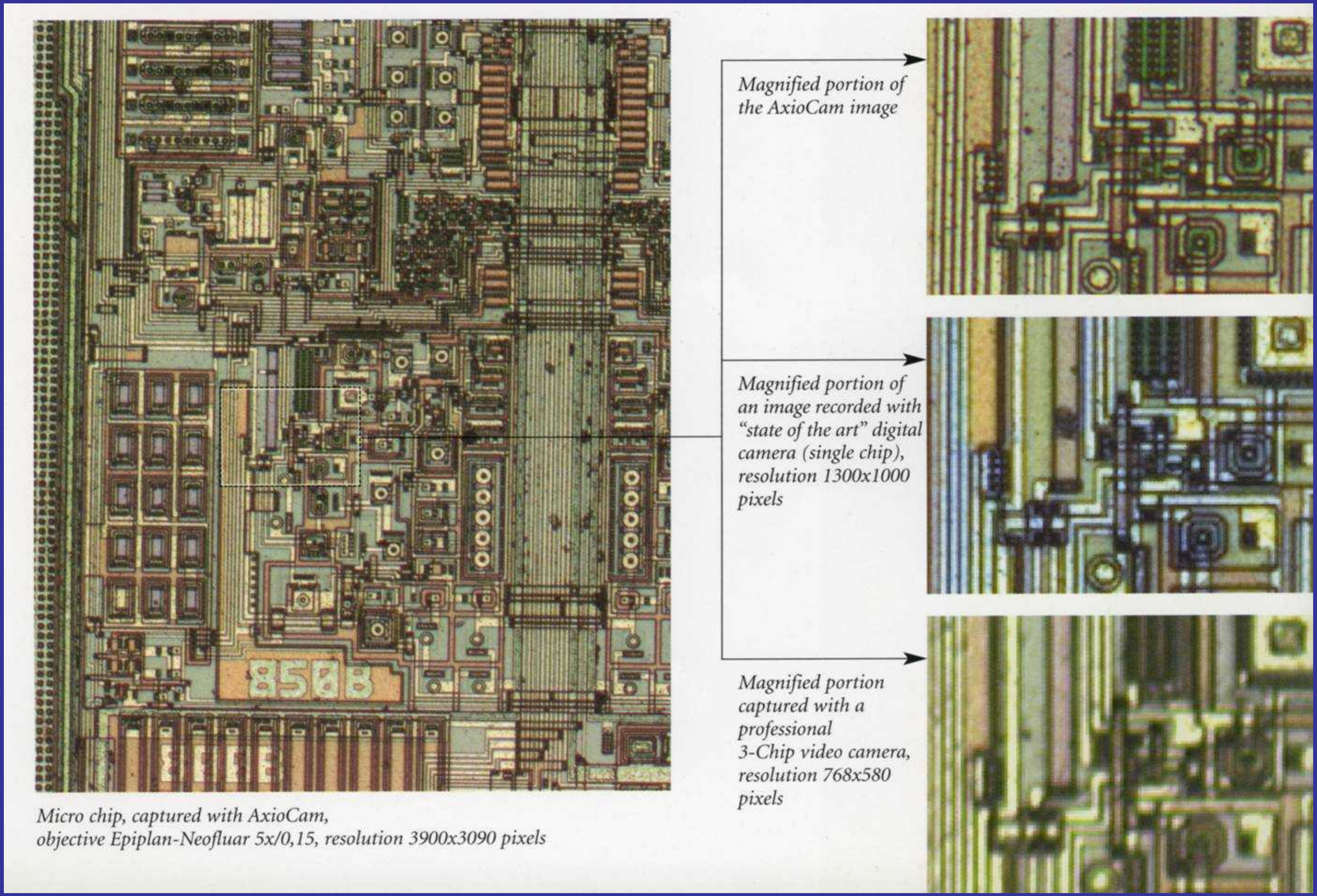
Factors affecting digital image quality.

Too few pixels, but large.

Right number and size

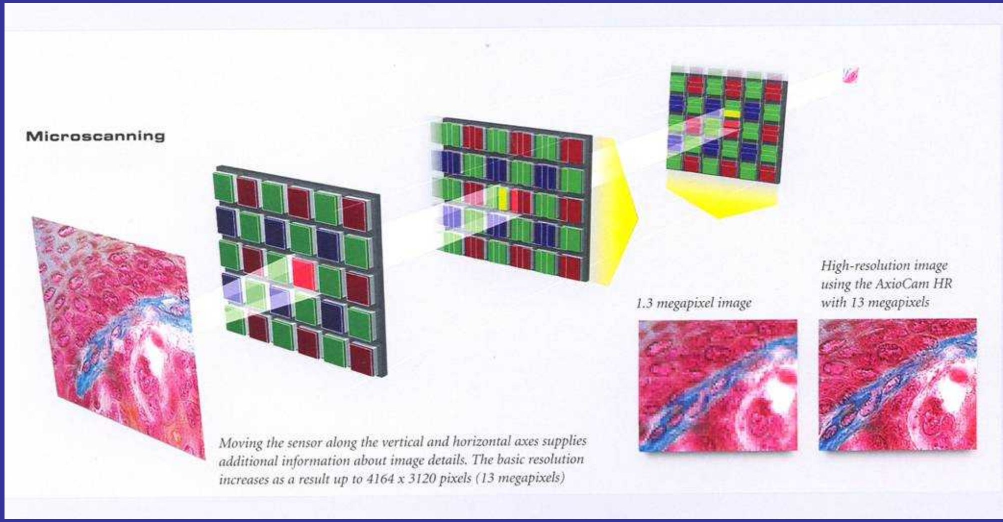
Pixels too small and noisy

Factors affecting digital image quality.



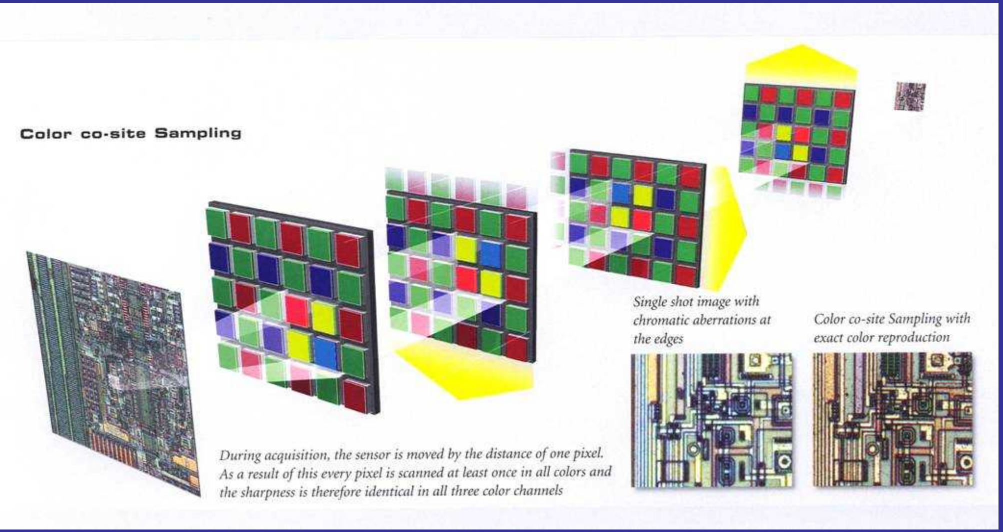
A way around! Large pixels and high resolution: Micro-scanning and Co-site sampling.

Factors affecting digital image quality.



Micro-scanning: using the spaces between pixels.

Co-site sampling: moving all three colours to the same location.



Advantages and disadvantages of different digital cameras.

- Compact camera with fixed lens.

- Pro. Inexpensive, dustproof.
- Con. May be difficult to attach.
  Lenses with more that 3:1 zoom ratio may not work.
  Difficult to operate shutter at the right time without causing vibration.
  Optical adapters variable quality, may hotspot.

Advantages and disadvantages of different digital cameras.

- Compact cameras with detachable lenses.

E.g., Sony NEX, Panasonic Lumix, Fuji, Leica.

Pro. Potentially allow image projection direct to sensor (be aware of need for compensating eyepieces), so higher image quality. Usually, larger sensors.

Con. Again, uncertain methods of attachment (T2?) and shutter operation.

Advantages and disadvantages of different digital cameras.

- <u>Single lens reflex cameras.</u> A mirror permits view through lens. When shutter tripped, mirror flips out and exposure made.

- <u>Pro.</u> Microscope manufactures make adapters, usually for the different sensor sizes. Largest sensors, potentially highest image quality.

- <u>Con.</u> Vibration from mirror. *Unless it has “Live View”.*

Advantages and disadvantages of different digital cameras.

- Digital camcorders.

- Mostly similar to compact cameras regarding attachment.
- Pro. Mostly inexpensive and lightweight.
- Con. Tiny sensor = poor still image and too high magnification.
- Best for movies: SLR with Full HD.

Advantages and disadvantages of different digital cameras.

- Mobile phone-type devices.

- Pro. Most have one, always ready.
  Fine for basic image quality/record shot.
  Con. At present, difficult to keep aligned etc.

Advantages and disadvantages of different digital cameras.

- Dedicated microscope cameras.

These consist of a case without any external controls. All control is from a computer.

Con. Can’t be used for much else except a telescope.

Low pixel number.

Fairly expensive to extremely expensive.

Pro. No vibration.

Accurate control of white balance and maybe shading correction.

Possible to make accurate measurements and include a scale bar or calipers on the image.

Extendable for many imaging and analytical methods.

How many megapixels do I need?

- What final result do you need? I.e., record shot/postcard-sized print, display print, suitable for publishing or ultimate resolution?
- What objective(s) will you use?

- In an ideal world, you would aim at highest resolution and downsize for record shots (because you can't go <u>up</u> that list).

How many megapixels do I need?

The theoretical limit of the resolving capability of light microscopes is highly dependent on the objective. The following table shows the resolving power of Carl Zeiss objectives in the intermediate image for 0.63x and 1.0x TV coupling adapters in combination with a 2/3" CCD sensor (8.5 mm x 6.4 mm).

	Objective	Magnification	N.A.	Lp/mm (TV-Cpl 1.0x)	Necessary camera resolution	Lp/mm (TV-Cpl 0,63x)	Necessary camera resolution
1.	Plan-Neofluar	1.25	0.04	96	1632 x 1229	152	2584 x 1946
2.	Fluar	2.5	0.12	144	2448 x 1843	229	3893 x 2931
3.	Plan-Neofluar	5	0.15	90	1530 x 1152	143	2431 x 1830
4.	Achroplan	10	0.25	75	1275 x 960	119	2023 x 1523
5.	Fluar	10	0.5	150	2550 x 1920	238	4046 x 3046
6.	Plan-Neofluar	20	0.5	75	1275 x 960	119	2023 x 1523
7.	Plan-Apochromat	20	0.75	113	1921 x 1446	179	3040 x 2291
8.	Plan-Neofluar Multi-Imm.	25	0.80	96	1632 x 1229	152	2584 x 1946
9.	Plan-Neofluar	40	0.75	56	952 x 717	89	1513 x 1139
10.	Plan-Neofluar	40	1.3	98	1666 x 1254	155	2635 x 1984
11.	Plan-Apochromat	63	1.4	67	1139 x 858	106	1802 x 1357
12.	Epiplan-Neofluar	100	0.9	27	459 x 346	43	731 x 550
13.	Plan-Apochromat	100	1.4	42	714 x 538	67	1139 x 858

According to the chart, a 1 megapixel camera will capture all the resolution of the best 100x objective.

But to capture all of the detail resolved by a 2.5x objective, an 11.4 megapixel is required, or 12.3 Mp with HQ 10x.

How many megapixels do I need?

Further considerations.

1. You may not need to image all that 2.5x objective can resolve.

2. Although in theory a certain detail that can be resolved by the microscope is imaged by the camera, this may lead to a jagged/rough image of it. To make a smooth image, even of objects at the limit of resolution, more pixels are required.

3. If you hope to submit images for publication, the standard file size is 47.5 Megabytes. This equals an 12"x8" print at 450 dpi or 16 megapixels.

Adapting digital devices to microscopes.

1. Compact cameras. Firm attachment with adapter from microscope manufacturer.

Adapting digital devices to microscopes.

Adapters for compact digital cameras. The main part is really just a huge, long-working-distance eyepiece. The silver part bayonets on to the camera around the lens – the lens moves freely inside it.

Adapting digital devices to microscopes.

Custom-making: you may find a clamping piece like these – they fit beneath eyepiece. Best use a wideangle eyepiece designed for spectacle wearers. The front element of the camera lens should be very close to the eyepiece top lens. Best to have a camera with bayonet around lens (used for attaching filters etc).

Adapting digital devices to microscopes.

This tube could possibly be attached to the limb top in place of the bino, an eyepiece added at the top and your camera mounted above.

Adapting digital devices to microscopes.

These are adapters for c-mount cameras (cine and most CCTV). Note the lens inside the camera mount. This corrects the remnant lateral chromatic aberration normally corrected by a compensating eyepiece. If you are using any camera without its lens on a microscope requiring compensating eyepieces, then you need to correct this – or suffer colour fringing at the corners.

Adapting digital devices to microscopes.

It may be possible to adapt a photomicrography outfit designed for film by adding an SLR to the top of the shutter unit, leaving the shutter open on B or T and using the system controls for exposure.