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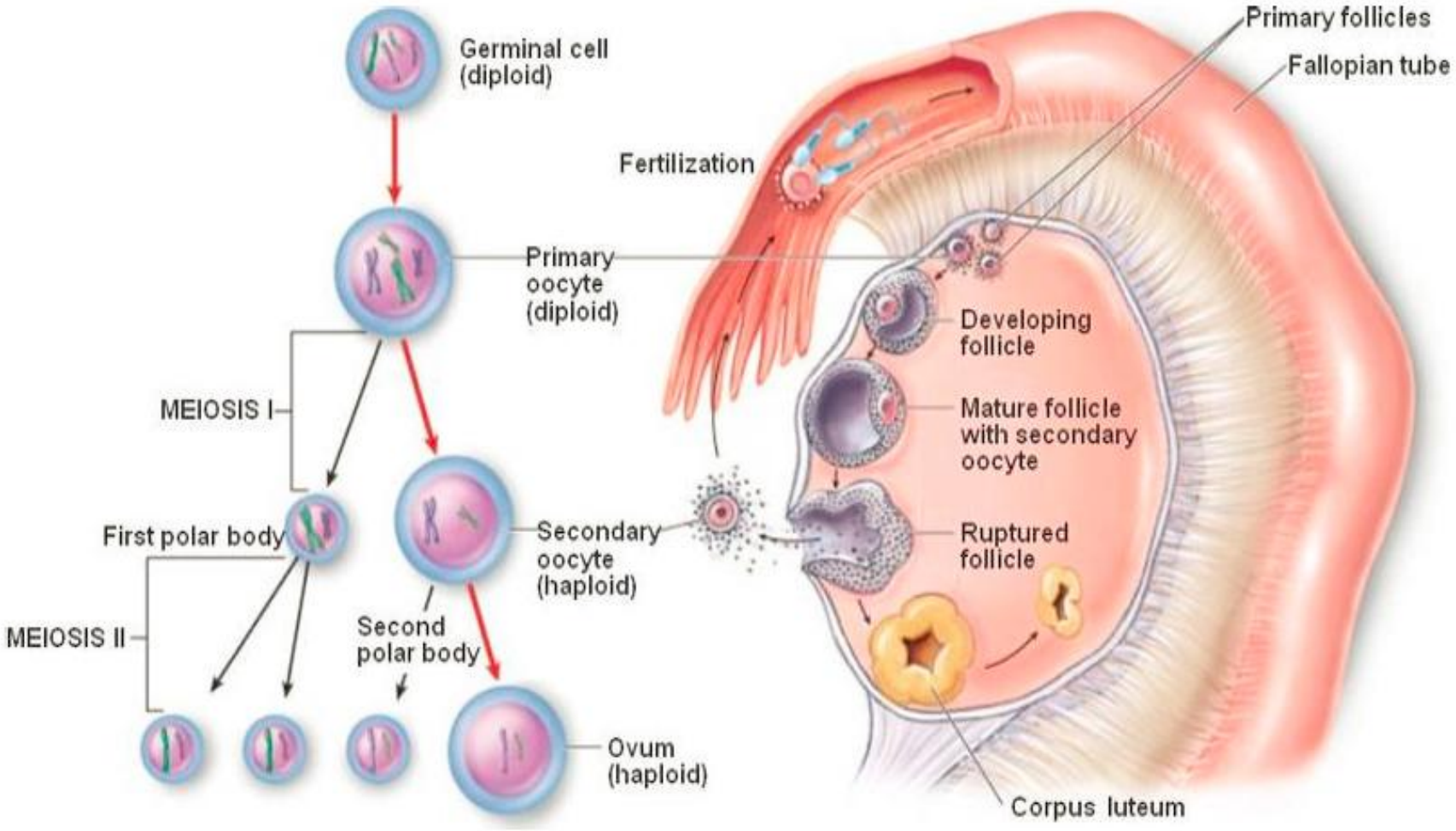
PLoS one

The Beneficial Effects of Antifreeze Proteins in the Vitrification of Immature Mouse Oocytes

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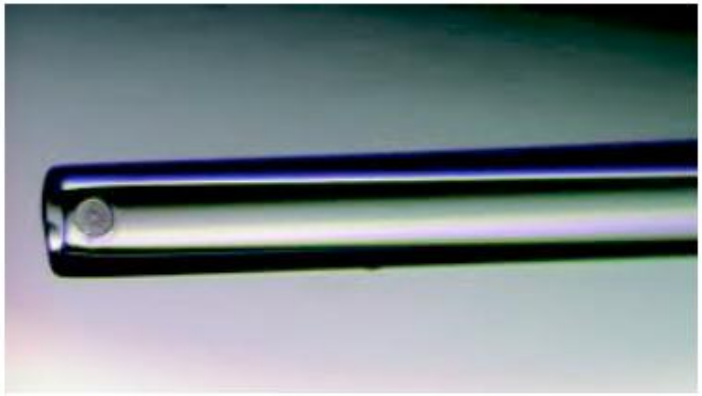
Maturation of Oocytes



http://buffonescience9.wikispaces.com/UNIT+3+-+Cell+Reproduction

Retrieval of Oocytes

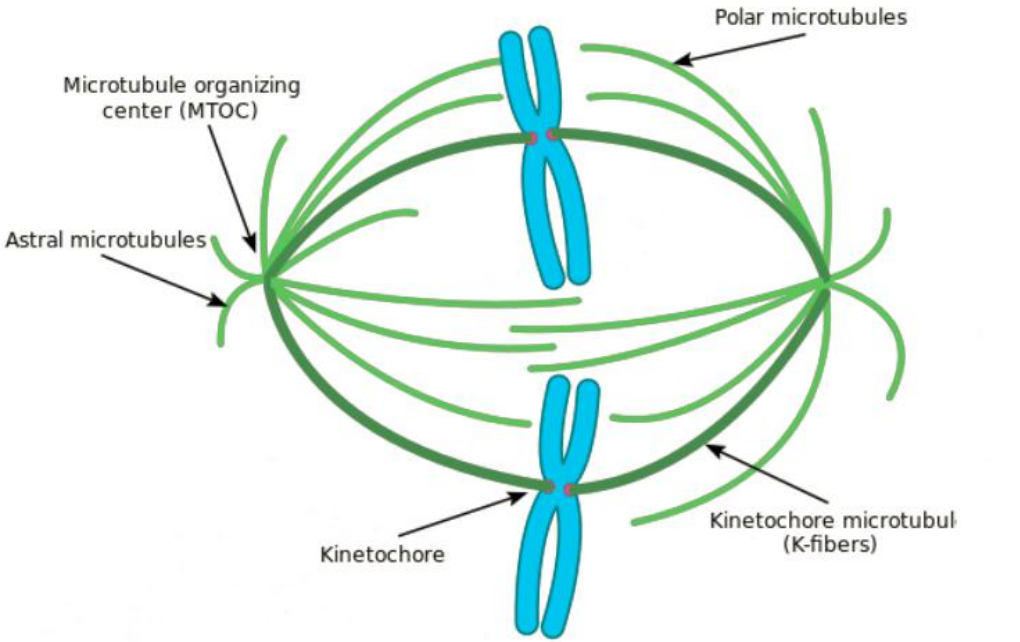




At what stage should we collect the oocyte?

http://www.kitazato.co.jp/biopharma/Cryotop1.html

Metaphase and Spindle Formation



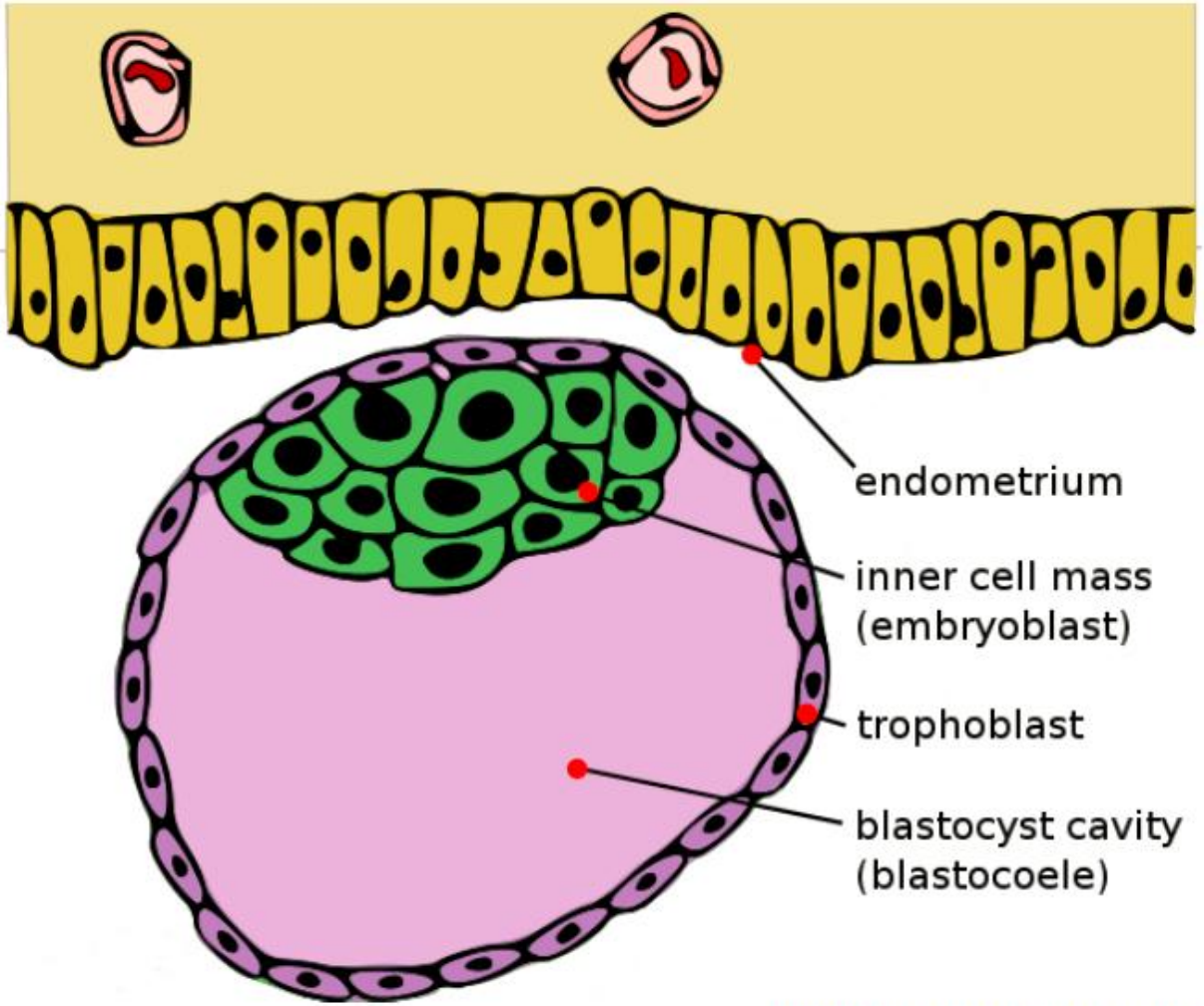
http://en.wikipedia.org/wiki/Spindle_apparatus

During Metaphase the chromosomes are exposed and lined up on the theoretical metaphase plate.

During prophase I the DNA has been duplicated, the nuclear envelope is still in tact.

Spindle Proteins: Mad2, Eg5 and Hook1

How functional are oocytes after freezing?



http://en.wikipedia.org/wiki/File:Blastocyst_English.svg

Frozen GVs have trouble maturing, cannot be fertilized, or are impaired during embryonic development.

In the paper the observe cleavage and blastocyst development.

CELLULAR CRYOPRESERVATION: AVOIDING INTRACELLULAR ICE FORMATION

1. **Supercooling** – taking a liquid below its equilibrium freezing point, without freezing



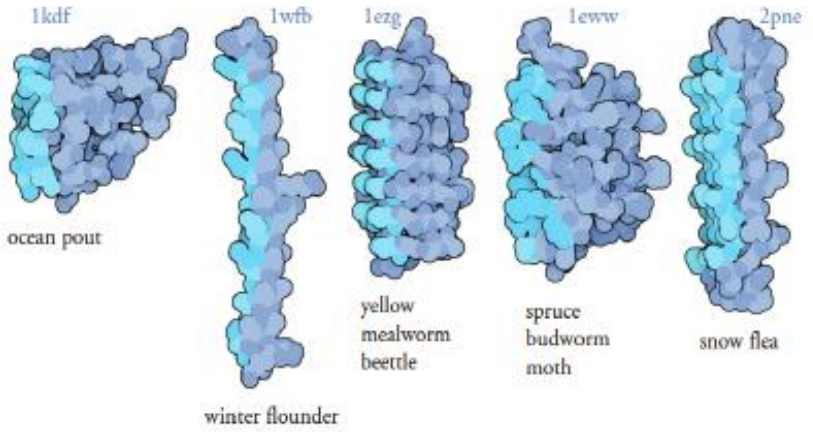


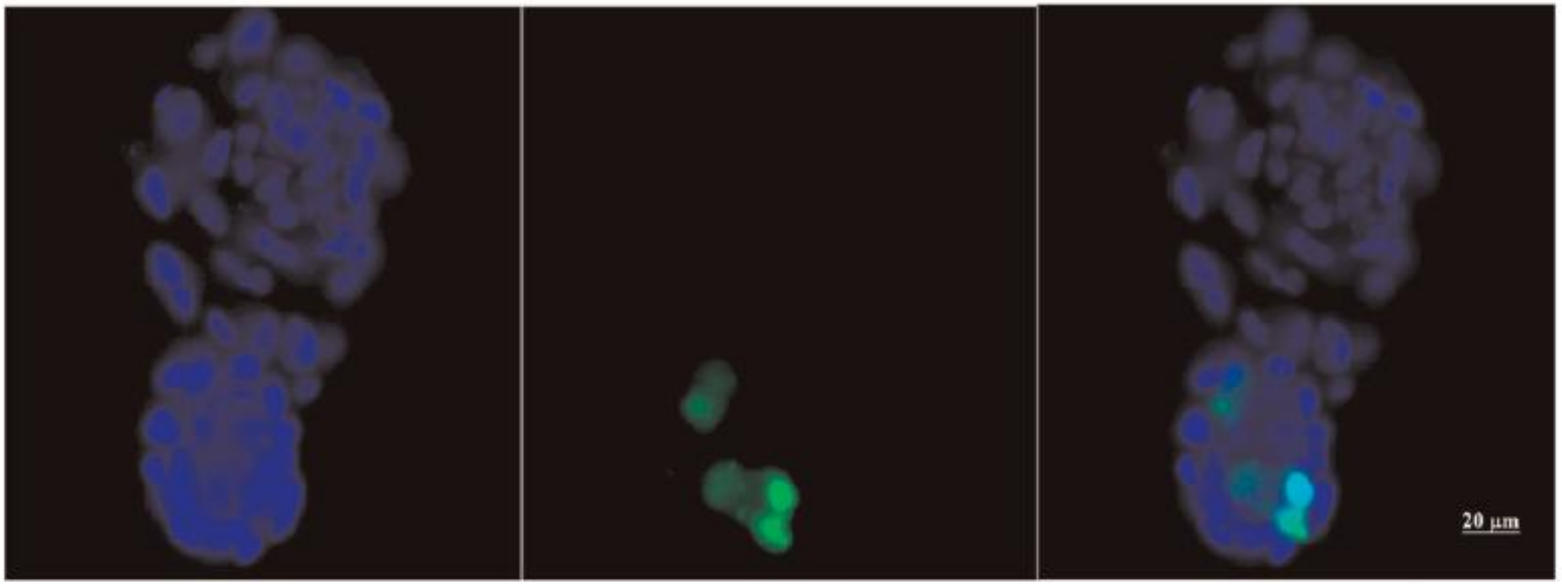
Table 1. Development of vitrified-warmed germinal vesicle (GV)-stage oocytes with or without antifreeze protein (AFP; 500 ng/mL) supplementation in vitrification media (seven replicates).

	AFP-treated	Untreated	P
Initiated GV oocyte	171	178	
Survived	166 (97.1%)	162 (91.0%)	0.017
Matured	129 (77.7%)	126 (77.8%)	NS
Cleaved	118 (91.5%)	88 (69.8%)	<0.01
Blastocyst (per cleaved embryo)	96 (81.4%)	59 (67.1%)	0.019
Blastocyst (per initiated GV)	96 (56.1%)	59 (33.2%)	<0.01
Blastomere count per blastocyst	78.2±11.8a	67.8±11.3b	<0.01
No. of caspase-positive blastomeres	285/3,987 (7.1%)	363/3,188 (11.4%)	<0.01
No. of trophectoderms per blastocyst	69.6±11.8c	54.0±5.3d	<0.01
No. of inner cell masses per blastocyst	13.9±2.2c	11.4±1.9d	<0.01

Mean±SD.
Number of blastocysts tested: a=51, b=47, c=23 d=22.
doi:10.1371/journal.pone.0037043.t001

Apoptosis in AFP vs Untreated?

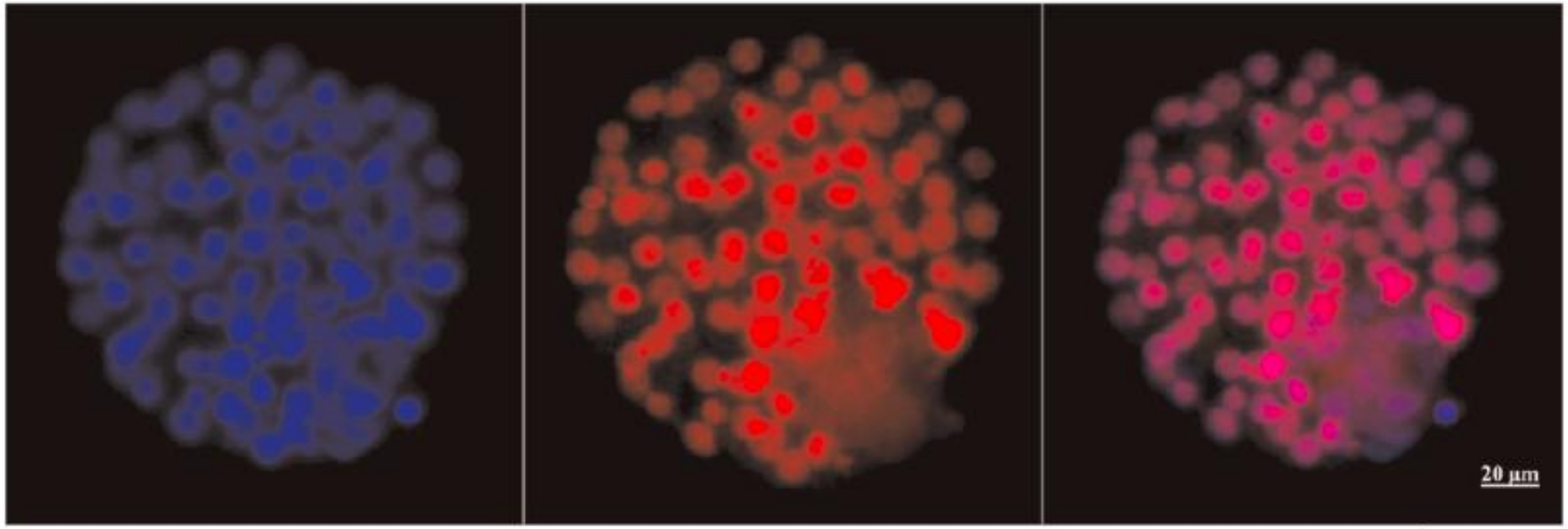
(I) Caspase positive blastomeres



a: DAPI b: FITC c: merged

Nuclear Stain of Blastocysts

(II) Blastocysts with differential staining



a: Hoechst 33342 b: PI c: merged

Grades of Spindle Formation

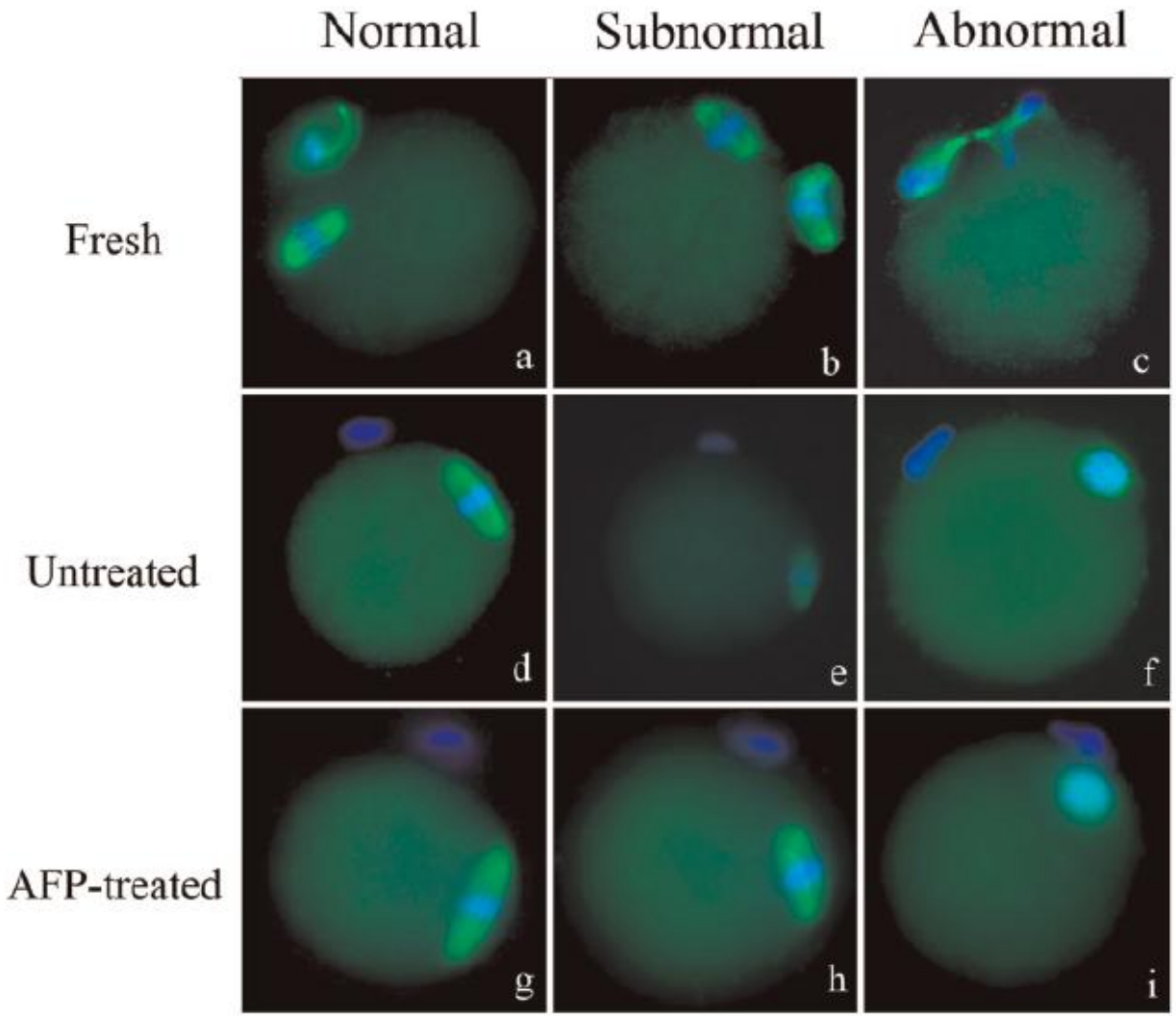
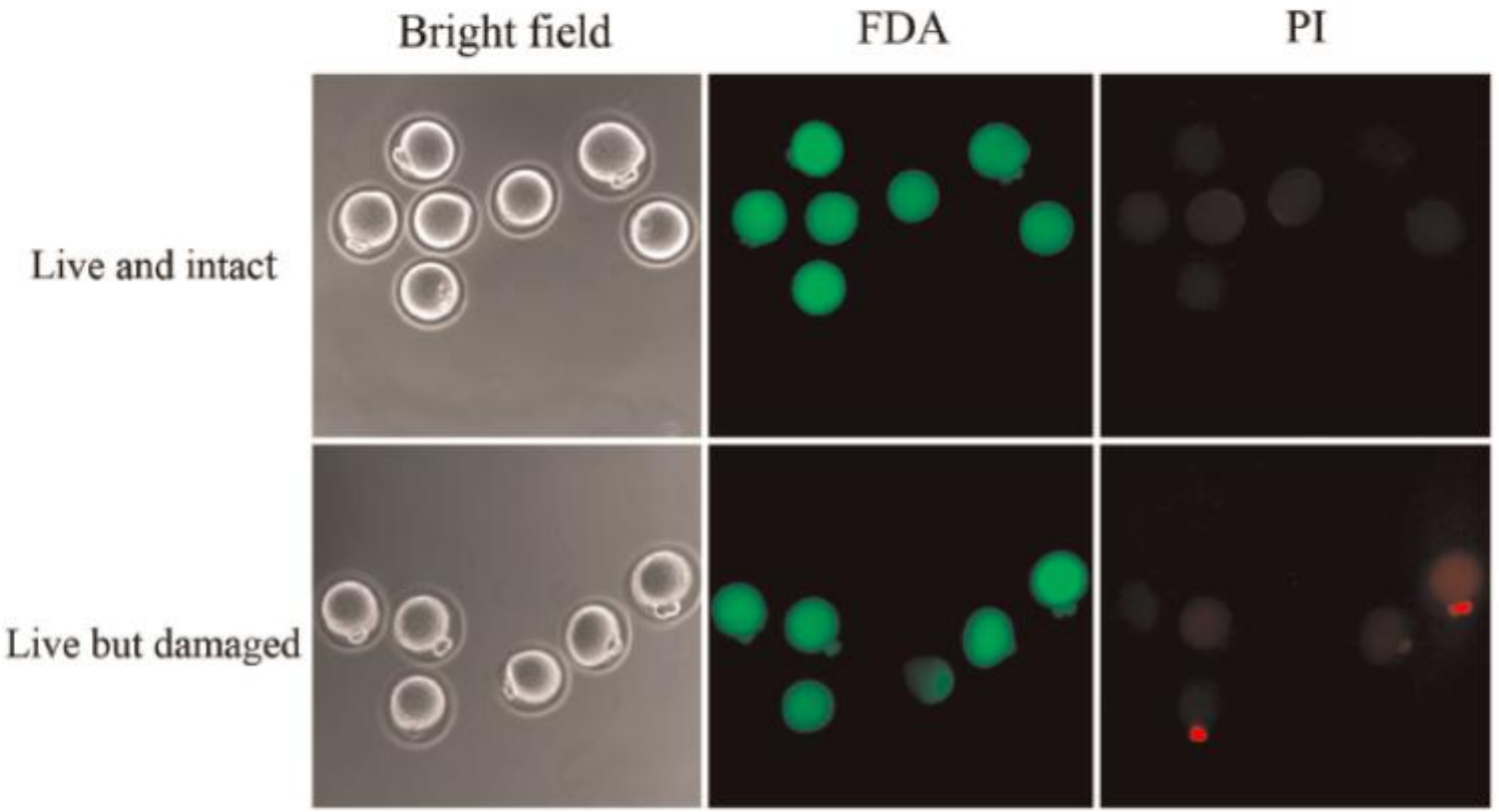


Table 2. Meiotic spindle organization and chromosome alignment of vitrified-warmed mouse oocyte supplemented with antifreeze protein (Five replicates).

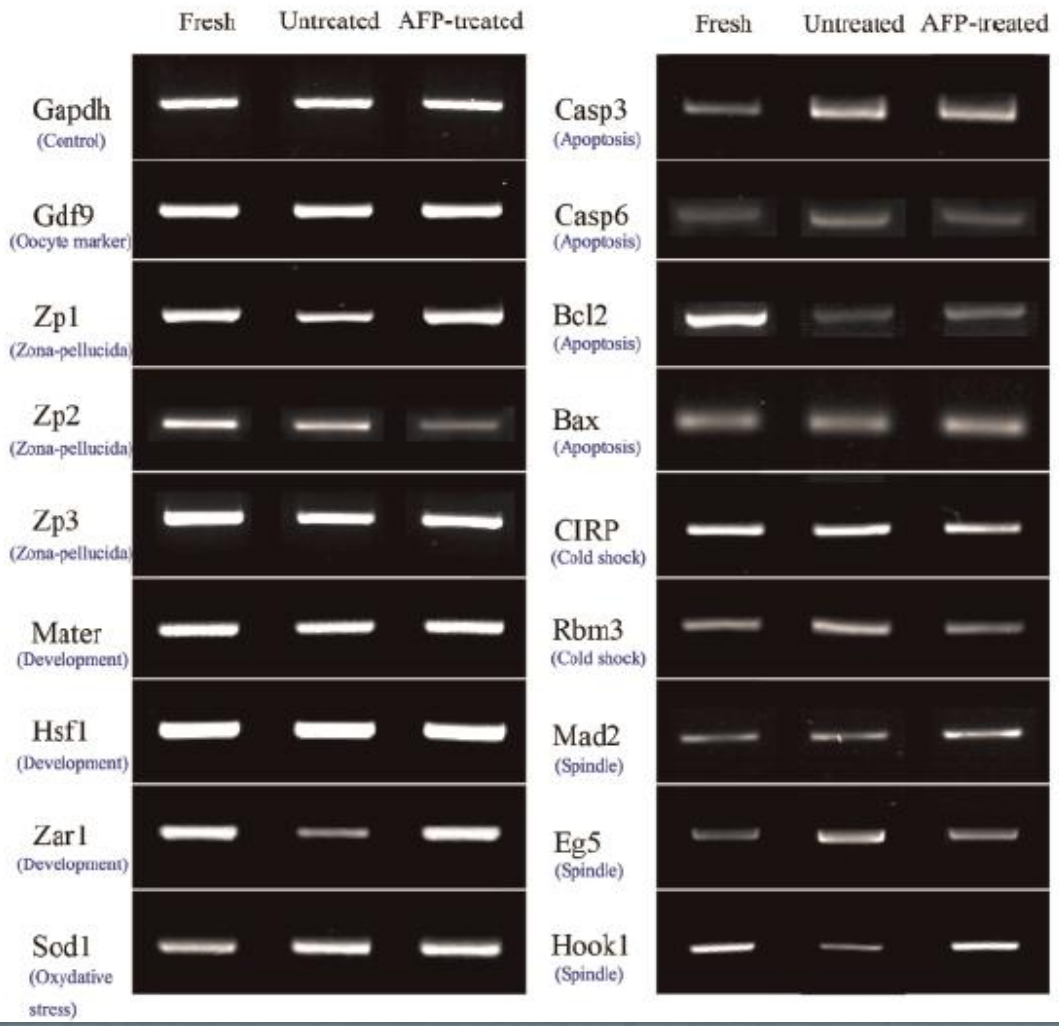
	Untreated	AFP-treated	P
No. of oocytes examined	88	78	
Normal	53 (60.2%)	61 (78.2%)	0.013
Sub-normal	21 (23.9%)	8 (10.3%)	0.021
Normal+Subnormal	74 (84.1%)	69 (88.5%)	NS
Abnormal	14 (15.9%)	9 (11.5%)	NS

doi:10.1371/journal.pone.0037043.t002

Membrane Integrity Tests



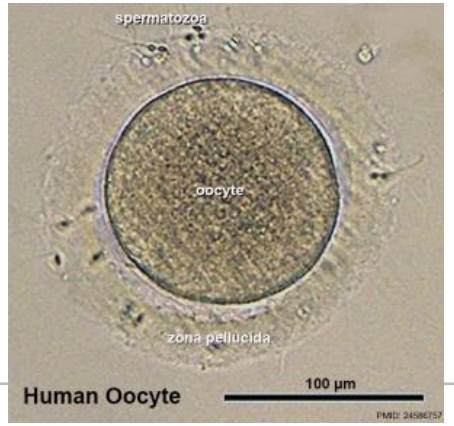
Mitosis and Meiosis Gene Marker Expression



In the AFP-treated group: Hook1, Zar1, Zp1 were similar to fresh

Zp2 was lower than fresh.

In the untreated Eg5 was elevated.



Thoughts?

- Significance in the Field?
- Was it thorough?
- Future directions?



Microscopy

MICROSCOPE (*n.)*

(from the Greek *mikrós*, "small" and *skopeîn*, "to look" or "see")
Def: An instrument used to see objects too small to see with the naked eye.

• First light microscope (essentially a magnifying lens) believed to have first been developed in the 1200s.

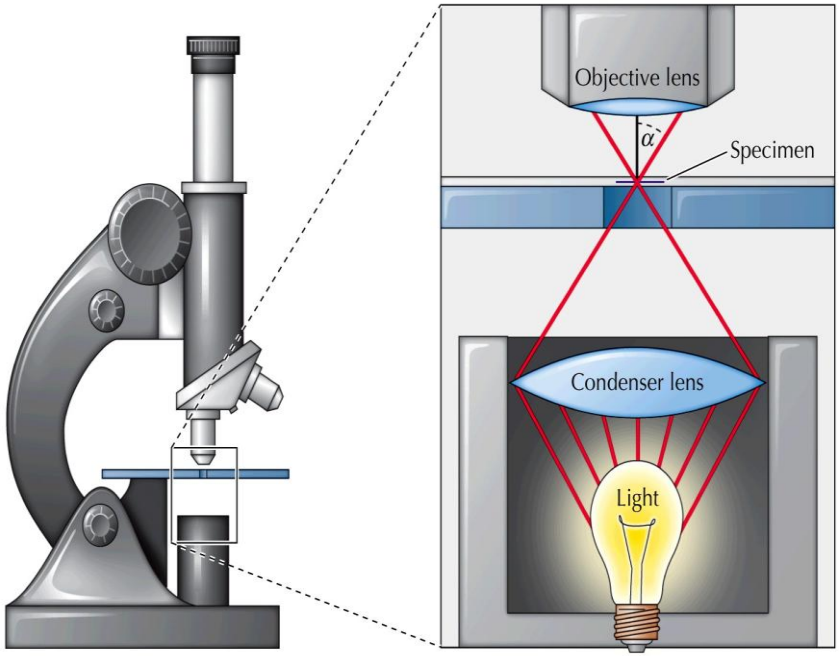
• Designed in their current **compound** form (use of multiple lens to magnify) in the 1600s. The inventor is unknown, however, Galileo Galilei and Antonie van Leeuwenkoek are often credited.



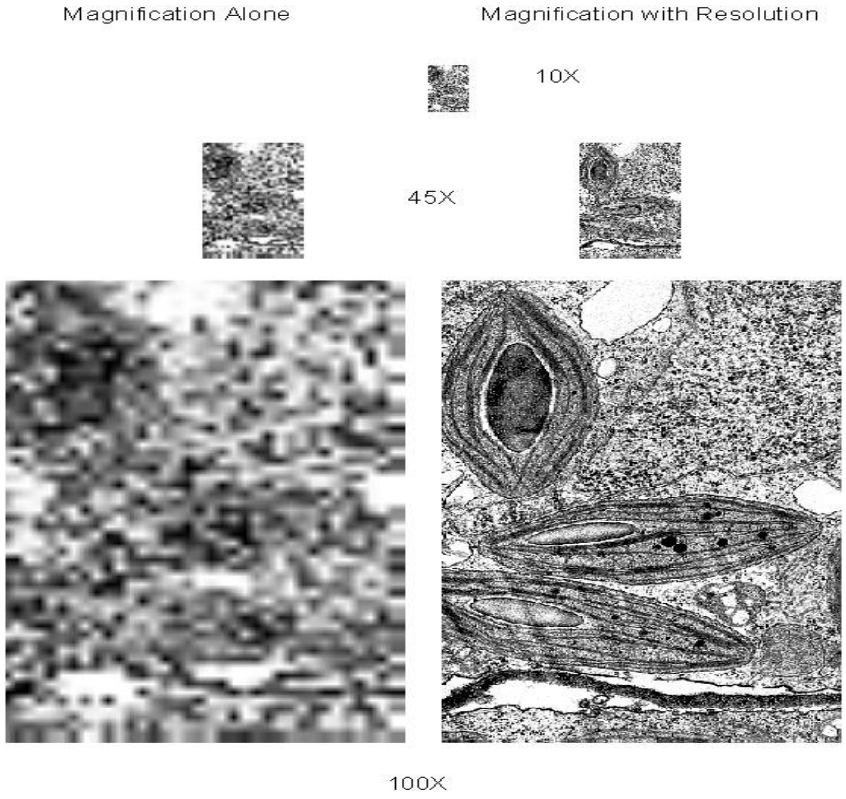
English: 18th century microscopes from the Musée des Arts et Métiers, Paris

LIGHT MICROSCOPY

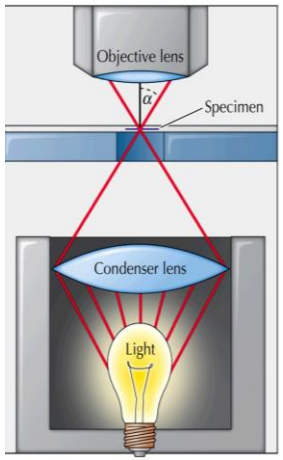
• Able to **magnify** objects up to 1000x. Sufficient for objects such as cells and large organelles (nuclei, mitochondria). The **resolution** power, however, of the light microscope limits its ability to observe the fine details of cell structures.



THE CELL: A MOLECULAR APPROACH 6e, Figure 1.23
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RESOLUTION



$$\text{Resolution} = 0.61\lambda/\text{NA}$$

λ= the wavelength of visible light (approx. 0.5um)
NA = numerical aperture
• The size of the cone of light that enters the microscope lens after passing through the specimen.

$$\text{NA} = \eta \sin \alpha$$

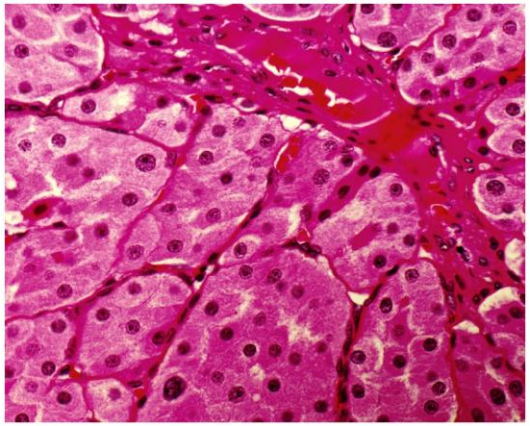
η= refractive index of the medium through which light travels between the specimen and the lens. ηfor air is 1.0, ηin oil immersion is 1.4
α= the angle of the cone of light collected by the lens

Limit of resolution for light microscopy is 0.22um

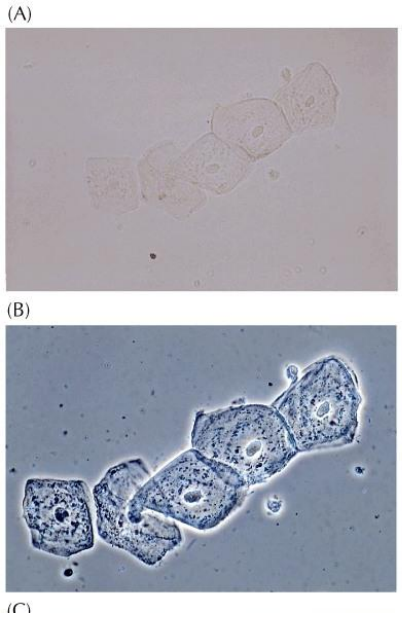
LIGHT MICROSCOPY

Bright-field microscopy: light passes through the cell and ability to see depends on the absorption of visible light by different cell components

- difficult to see cells (poor contrast) without fixative and staining



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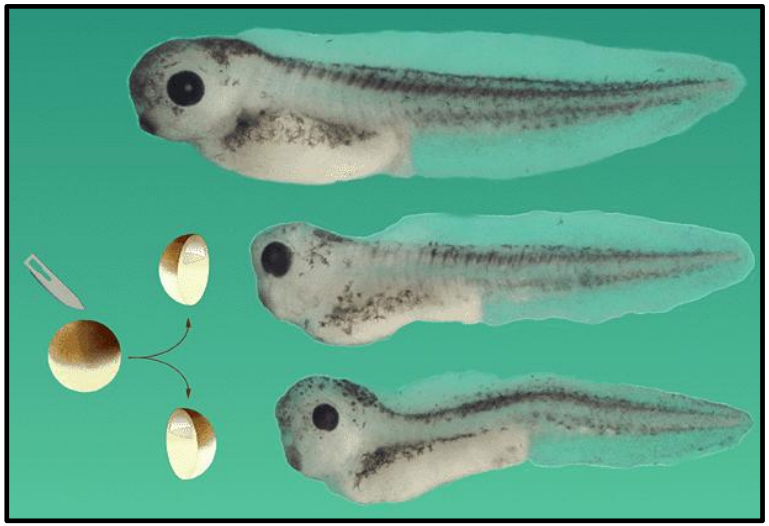
Phase-contrast microscopy: converts variations in thickness to differences in contrast that can be seen in the final image

- allows for imaging of live, unstained cells

LIGHT MICROSCOPY

Dissecting microscope: designed for low magnification of a specimen with three dimensional detail. Light is reflected off the surface, rather than passing through it, and an image is generated through two optical paths – one for each eye. This produces a three-dimensional, or stereoscopic image.

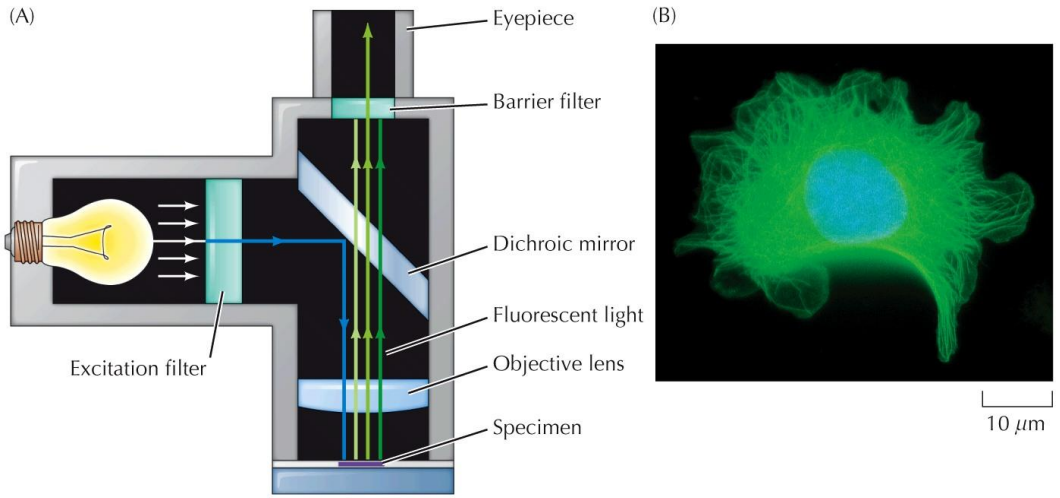
- Used extensively in micro-dissection





LIGHT MICROSCOPY

Fluorescence microscopy: use of a fluorescent dye to label molecules of interest within a cell. The dye absorbs light at one wavelength and emits at a second. The specimen can then be illuminated by the "excitation" wavelength and filters use to detect the "emission" wavelength.



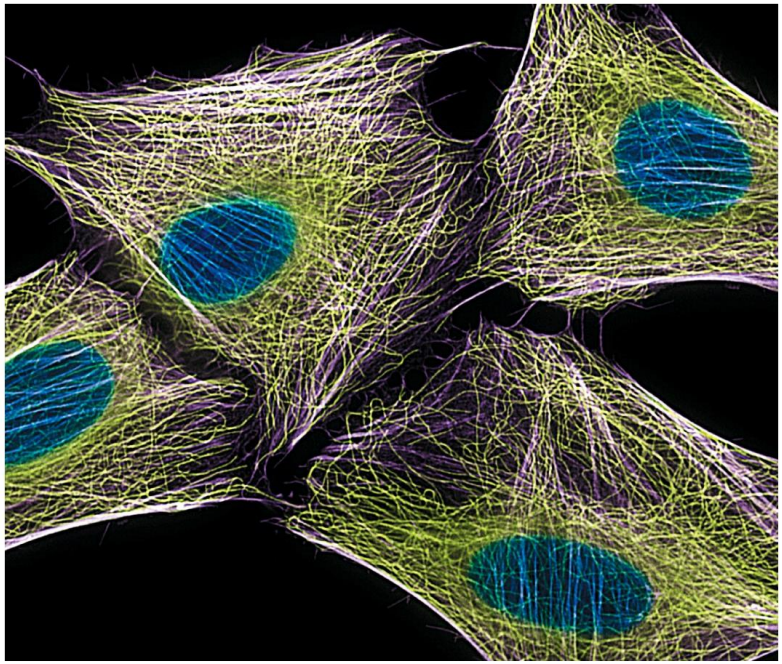
THE CELL: A MOLECULAR APPROACH 6e, Figure 1.27
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Green fluorescent protein (GFP)

Protein expressed by the jellyfish *Aequorea victoria* and first used as a fluorescent marker in the 1990s. GFP can be used as a reporter of expression of a protein of interest using recombinant DNA techniques.

LIGHT MICROSCOPY

Confocal microscopy: allows images of increased contrast and detail to be obtained by analyzing fluorescence from only a single point in the specimen. Produces a much sharper image than standard, widefield, fluorescence microscopy



***THE CELL: A MOLECULAR APPROACH 6e*, Figure 1.32**
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ELECTRON MICROSCOPY

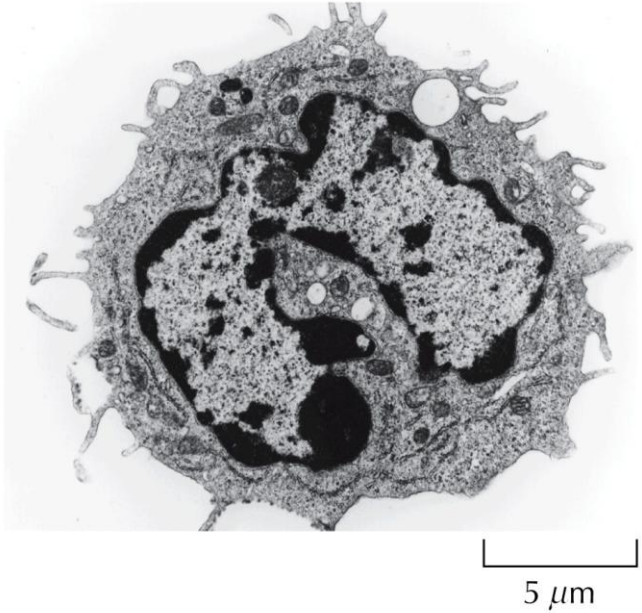
- Use of electrons instead of light to pass through the specimen

- Allows for much higher resolution due to the short wavelength of electrons (0.004nm – 100,000x shorter than visible light)

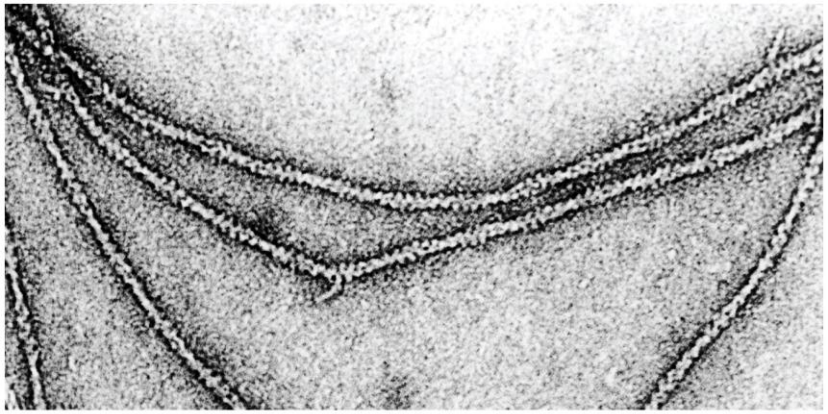
Limit of resolution for electron microscopy (with biological samples) is 1 to 2nm. ***More than 100x improvement over light microscopy.***

ELECTRON MICROSCOPY

Transmission electron microscopy: similar to the bright-field light microscope in concept, only using electrons instead of light. Specimen are stained with heavy metals which deflect electrons and will appear black.



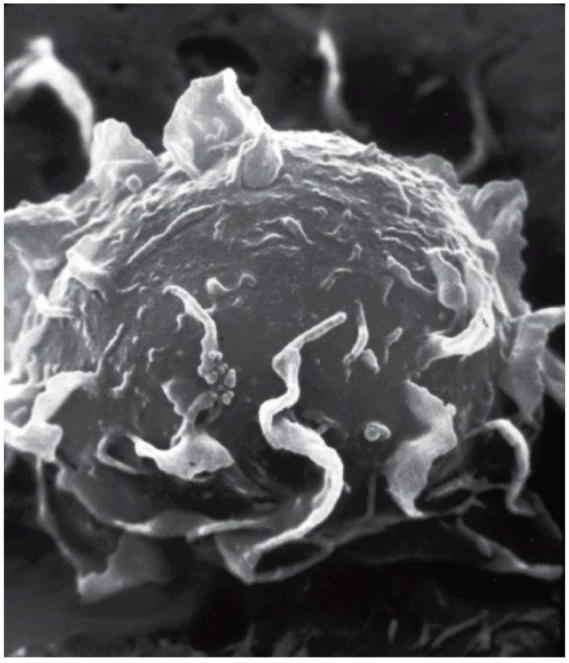
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ELECTRON MICROSCOPY

Scanning electron microscopy: The surface of the cell is coated with heavy metal and electrons are bounced off the specimen (like in a dissecting scope) to create a three-dimensional image.



***THE CELL: A MOLECULAR APPROACH 6e*, Figure 1.38**
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USE AND CARE OF THE MICROSCOPE
inverted, phase-contrast

• Remember: magnification is the objective lens (4x, 10x, 20x, 40x) multiplied by the eye-piece (10x)

• Always turn off the light when done and replace the dust cover

• Use the appropriate phase shift ring as indicated on the objective lens (4x = Ph0 10x, 20x, 40x = Ph1 20x, 40x = Ph2)



USE AND CARE OF THE MICROSCOPE
EVOS

- Digital inverted microscope for use in bright-field, phase-contrast and fluorescence microscopy.
- Be sure that the phase annuli selector is in the correct position that corresponds to the selected objective and contrast method.
- Use the light shield box to reduce ambient light and improve image quality.
- Bring a USB flash drive if you'd like to save images for lab reports.
- Always remember to turn off the microscope and replace the dust cover when done.

