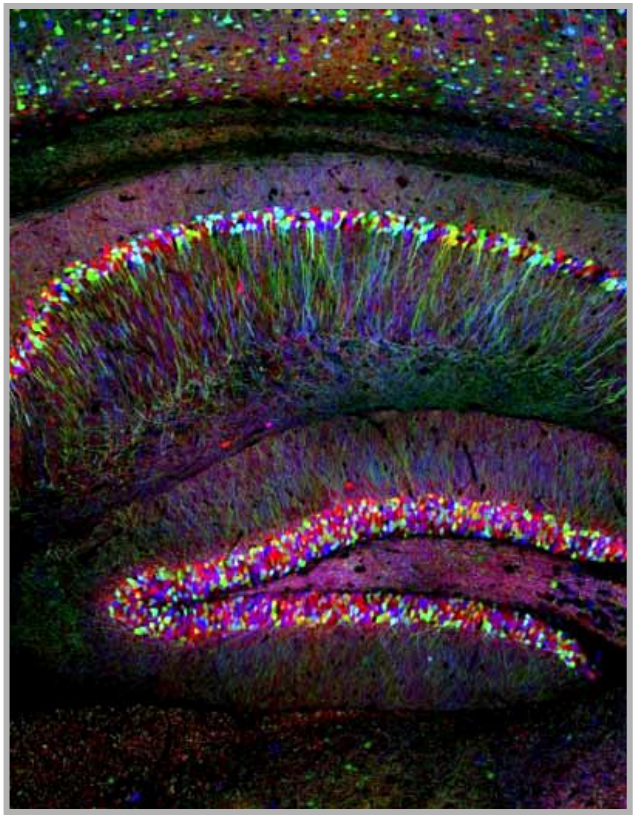
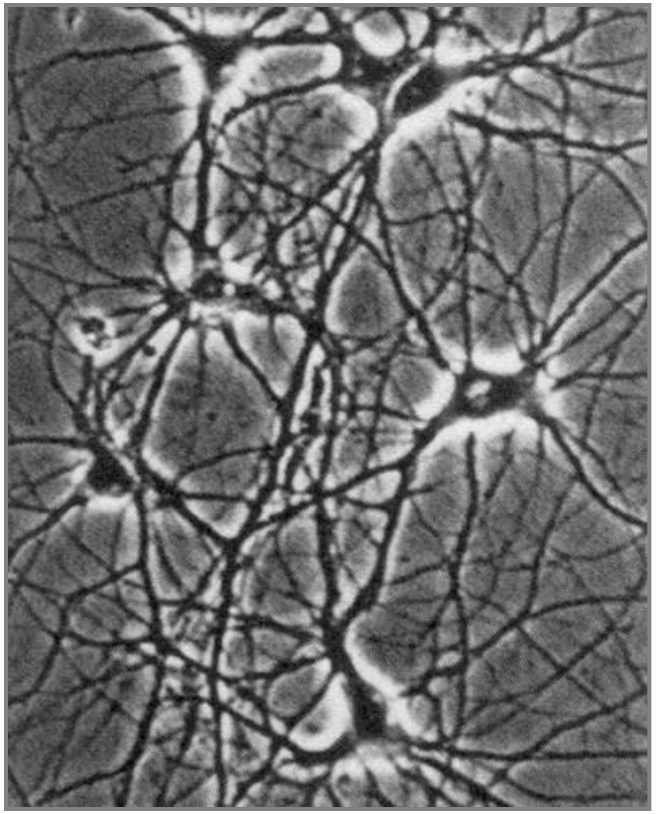
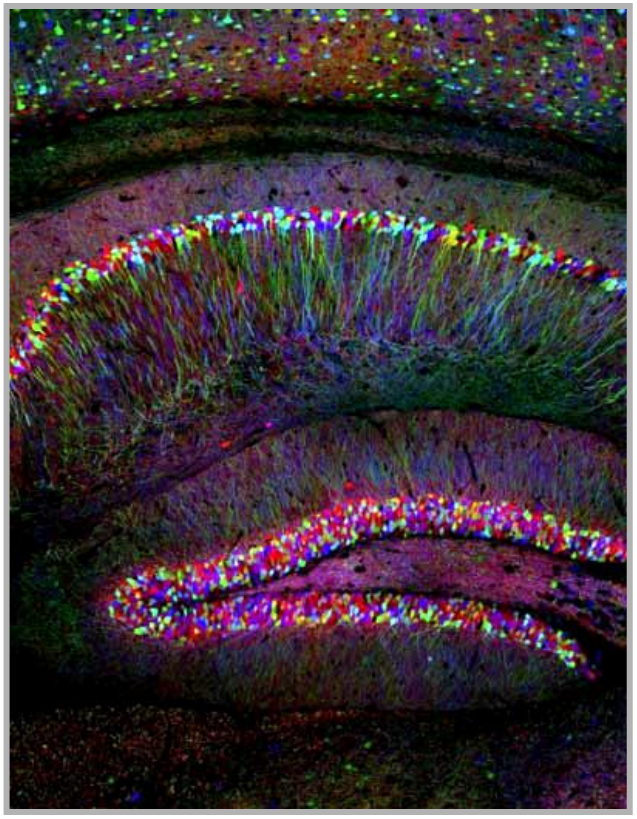
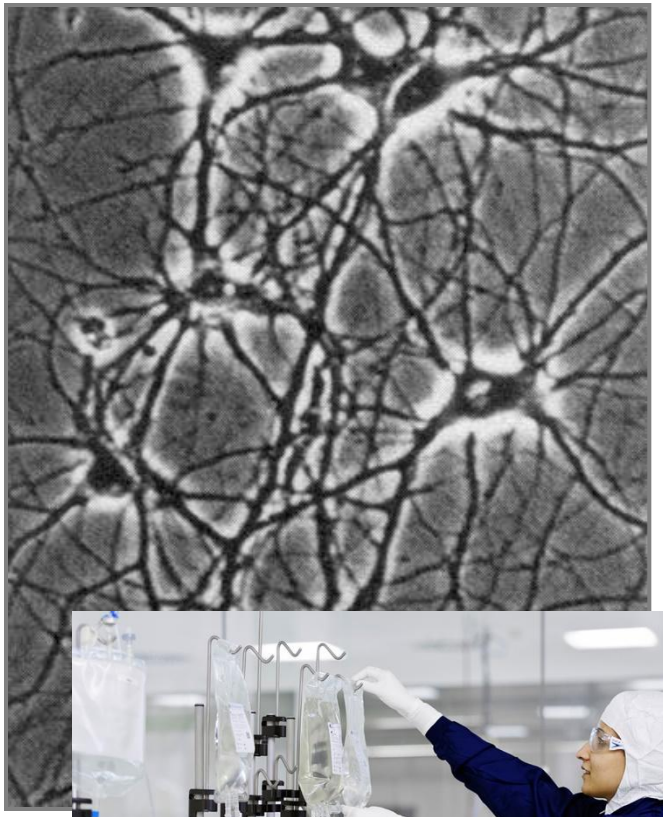
Cell culture





Cell culture





Cell culture

Year	Event
1885	**Roux** demonstrates that chick cells can be kept alive in saline
1907	**Harrison** cultures frog spinal cord cells on clotted lymph, basis of neuronal doctrine
1913	**Carrel** established extended cultures through regular feeding under aseptic conditions
1948	**Earle**: single cells isolated and cultured, giving rise to the L cell line
1950s	initial use of serum in media
1952	**Gey** established HeLa cell line from a human cervical carcinoma
1955	**Eagle** systematic investigation into nutrient formulations.
1958	**Temin** and **Rubin** viral transformation of chick cells accomplished in the laboratory
1964	**Kato** and **Takeuchi** grow complete carrot from single plant cell
1965	**Harris** and **Watkins**: viral-induced fusion of mouse and human cells
1975	**Köhler** and **Milstein**: first monoclonal antibody-secreting hybridoma cell lines
1976	**Sato** demonstrates in serum-free culture that different cells need different media
1970's	**Boyer** and **Cohen** develop recombinant DNA-technology
1977	**Wigler** and **Axel** introduce mammalian genes into cultured cells
1986	**Martin** and **Evans** isolate and culture mouse embryonic stem cells
1998	**Thomas** and **Gearhart** isolate human embryonic stem cells

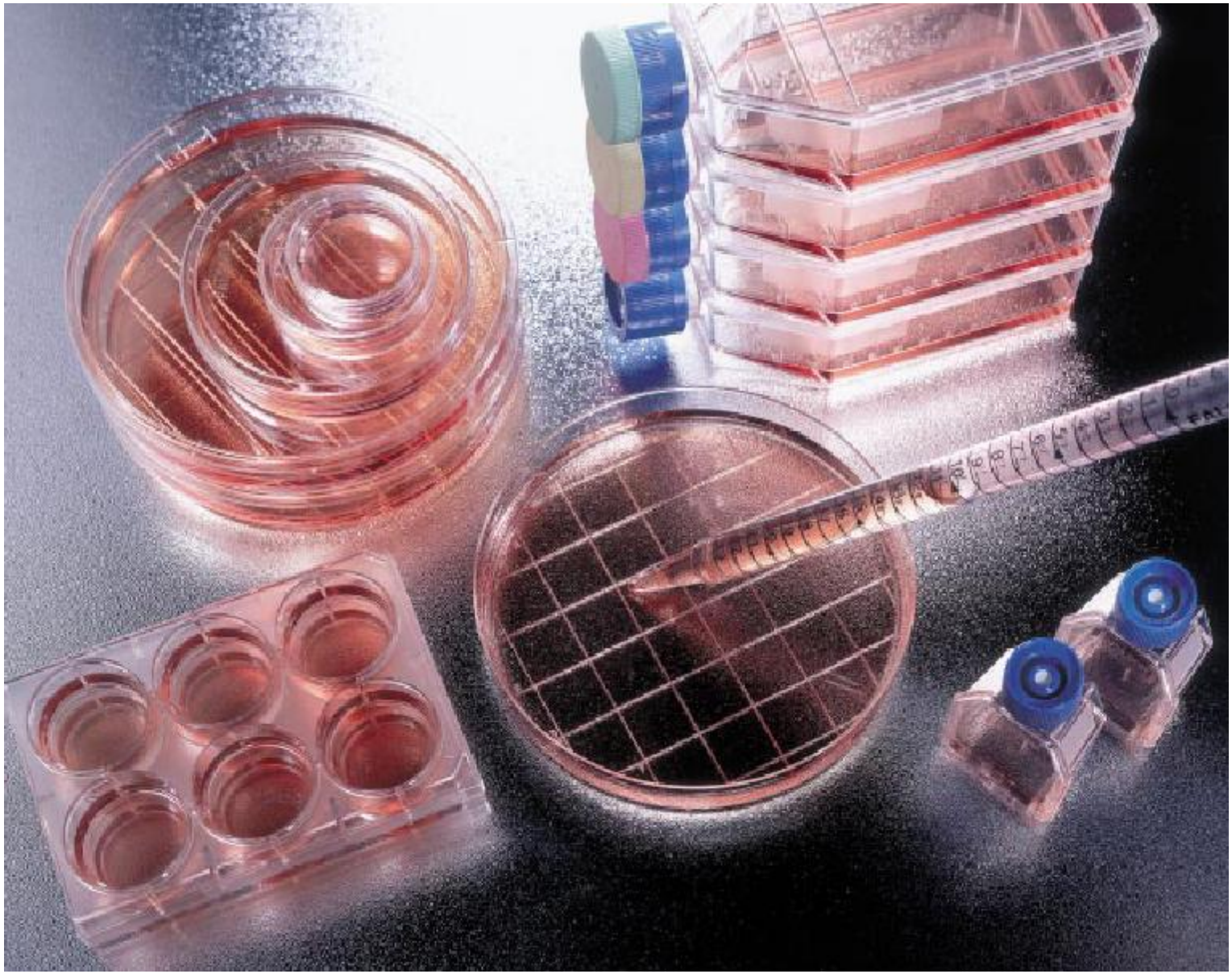
Cell culture - challenges

Equipment / techniques of cell culture seek to replicate and control specific aspects of the tissue environment:

- Temperature
- pH
- Nutrients delivery
- Waste removal
- Anchorage

- Experimental observation and manipulation

Modern materials



Cell culture media – Dulbecco's Modified Eagle's Medium

Amino acids (mg/L)

- L-Arginine•HCl (84.0)
- L-Cystine (48.0)
- L-Glutamine (584.0)
- Glycine (30.00)
- L-Histidine•HCl•H_2O (42.0)
- L-Isoleucine (104.8)
- L-Leucine (104.8)
- L-Lysine•HCl (146.2)
- L-Methionine (30.0)
- L-Phenylalanine (66.0)
- L-Serine (42.0)
- L-Threonine (95.2)
- L-Tryptophan (16.0)
- L-Tyrosine (72.0)
- L-Valine (93.60)

Other components (mg/L)

- Glucose (4500)
- Phenol Red (15.0)
- Sodium Pyruvate (110.00)

Salts (mg/L)

- $CaCl_2$ (200.0)
- $Fe(NO_3)_3$•$9H_2O$ (0.1)
- KCl (400.0)
- $MgSO_4$•$7H_2O$ (200.0)
- NaCl (6400.0)
- $NaHCO_3$ (3700.0)
- NaH_2PO_4•H_2O (125.0)

Vitamins (mg/L)

- D-Ca Pantothenate (4.0)
- Choline Chloride (4.0)
- Folic Acid (4.0)
- *i*-inositol (7.0)
- Nicotinamide (4.0)
- Pyridoxine•HCl (4.0)
- Riboflavin (0.4)
- Thiamine•HCl (4.0)

Cell culture media – serum

- Cell culture media (DMEM, Eagle, RPMI) is not enough to get cells to grow.
- Serum, isolated from clotted blood, was a critical discovery leading to modern cell culture
- Serum is added at some fraction of the cell culture media.
- Serum is a mix of proteins, carbohydrates, vitamins,minerals, much more.

Cell culture media – serum

Full understanding of what each component does is not clear, but some important players are:

- Growth factors (<100 ng/ml, each):
  - Epidermal Growth Factor (EGF), Platelet-derived Growth Factor (PDGF), IGF-1, IGF-2, FGF, IL-1, IL-6
- Hormones (<100 ng/ml each):
  - Insulin - promote uptake of glucose and amino acids
- Other components (which are particularly important for this class):
- Albumin (20-50 mg/ml) – lipid transport. The "globular protein". Non-cell adhesive
- Fibronectin (1-10 μg/ml) – cell adhesive protein

Cell culture media – serum

Common sources:

- Fetal Bovine Serum (FBS) – the classic serum, good growth characteristics
- Calf Serum (CS) – not as full of growth factors, cheaper
- Other species – chick, mouse, sheep, human

Drawbacks of serum:

- natural product
  - immense batch to batch variability
  - unknown components
  - availability
- relatively short shelf life
- growth inhibitors

Alternatives to serum are constantly being developed. Currently, no "silver bullet" exists.

Cell culture media – focus on pH

$$\mathrm{pH} = -\log[H^+]$$

bicarbonate buffering system

$$HCO_3^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$$

bicarbonate (soluble)	carbonic acid (soluble)	carbon dioxide (gas)

- Carbon dioxide dissolved in media and in the atmosphere regulate pH
- Primary mechanism of pH regulation in animal tissues
- Phenol red in the media changes color with respect to pH.
  - Orange-red at correct pH.
  - Purple at high pH
  - Yellow at low pH (*e.g.* in the presence of a lot of lactic acid

Cell culture incubators – pH and temperature

Cell culture incubator

- Maintain temperature
  - 37° C for most mammalian cells
- Maintain humidity
- Maintain 5% CO_2/air mixture
  - Bicarbonate buffer
  - pH 7.4

Outside the incubator

- Relatively easy to control temperature
- Control of pH ~ 7.4
  - bicarbonate for closed system
  - HEPES buffer - good
  - Tris buffer – okay

MCO-20AIC with world recognized SANYO Electronics microprocessor control. Shown with five adjustable shelves, included.

This all acts in concert with cell growth geometry

Cell culture substrates

Anchorage dependence

- Tissues are communities of cells – support framework
- Many cells are structural components
- Most cells types isolated from animal sources are anchorage dependent
- Exceptions
  - bacteria, fungi, yeasts, *etc*.
  - Cells normally found in circulating blood – T cells, B cells
  - Abnormal cells, now used as protein factories

The original – glass!

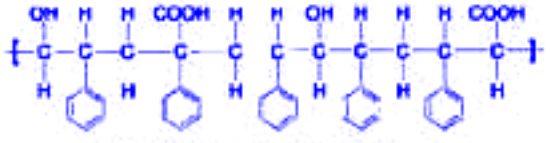
- Thus, *in vitro*
- Still the choice for routine microscopy applications

This is the new stuff – plastics, mostly polystyrene

- “Disposable”
- Quite good quality control (varying on a vendor basis) gives a predictable surface
- Myriad shapes
- Largely fluorescent, bad for microscopy.

Cell culture substrates

Polystyrene



- As cast, hydrophobic – this is a classic Petri dish
- Surface treated to become hydrophilic (gas plasma, irradiation, *etc.*)

Anchorage dependent cells:

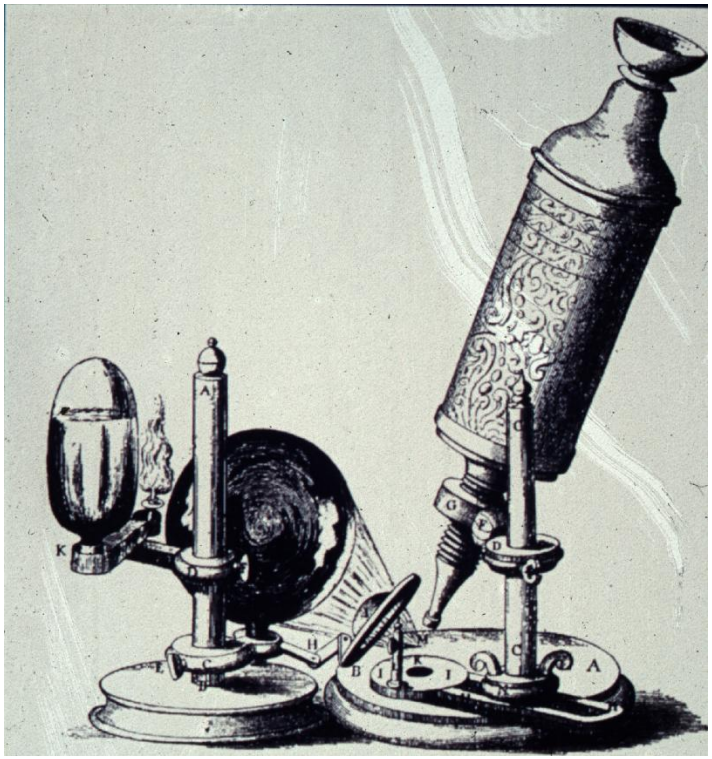
- attach and grow on hydrophilic surfaces
- loosely attach, have trouble growing on hydrophobic surfaces

The mechanism is not in the surface itself, but in the layer of proteins that adsorbs onto the surface, which the cells then recognize. Thus:

- Competitive absorbance of albumin and fibronectin from serum onto standard cultureware is a strong determinant of cell growth
- Coating the surface with a known protein or compound is used to both enhance cell growth and learn what proteins the cells see in the body

Systems for high-volume growth

Microscopy



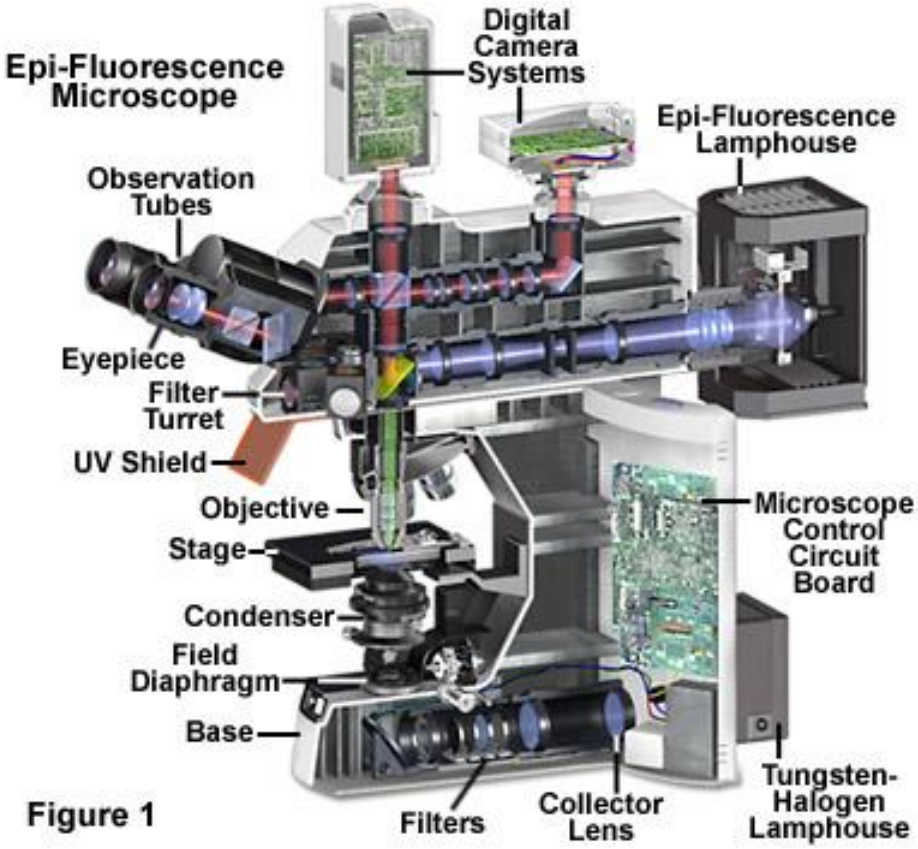


Figure 1

Light microscopy resources

- www.microscopyu.com
- http://www.olympusamerica.com/seg_section/seg_home.asp?CMP=ILC-homemicroscope (click on “Microscopy Resource Center”)
- www.probes.com

Phase contrast microscopy

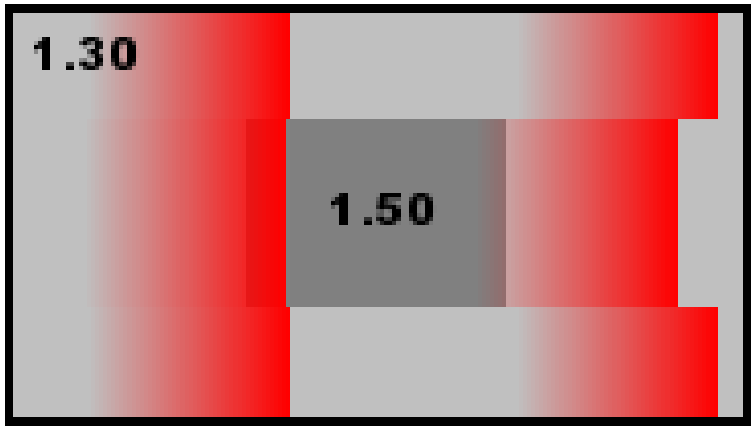
Brightfield

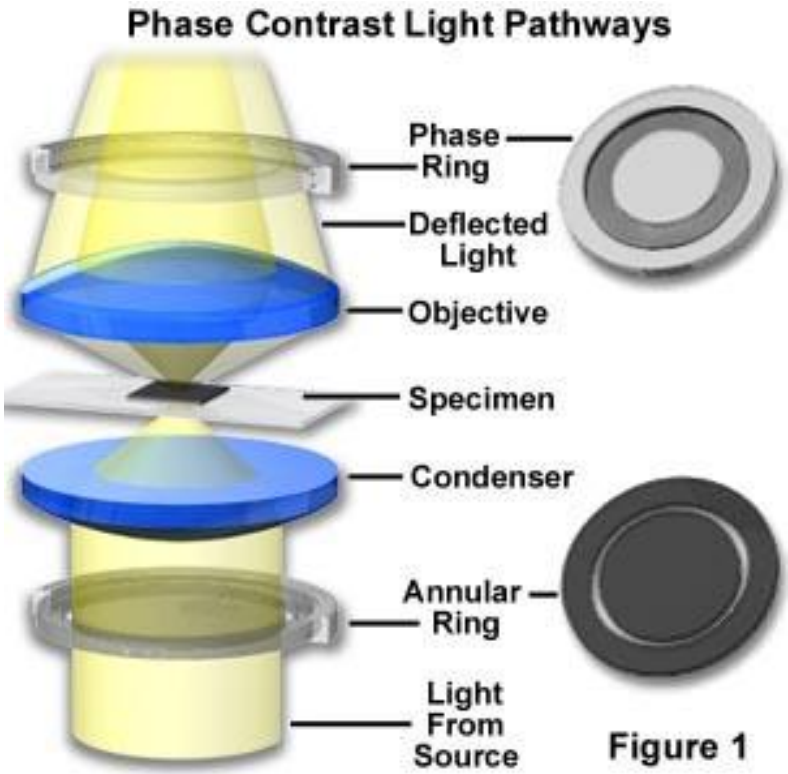
Phase contrast

Phase contrast microscopy

Brightfield

Phase contrast



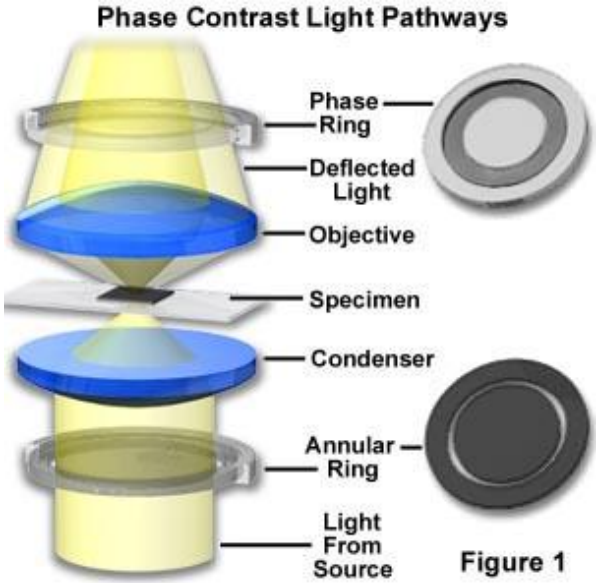


Phase contrast microscopy

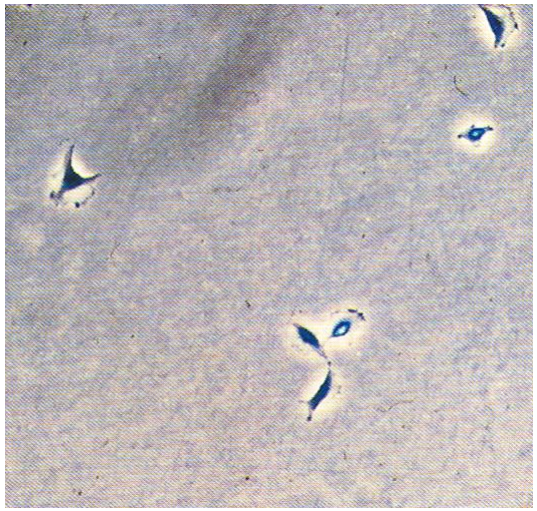
Brightfield

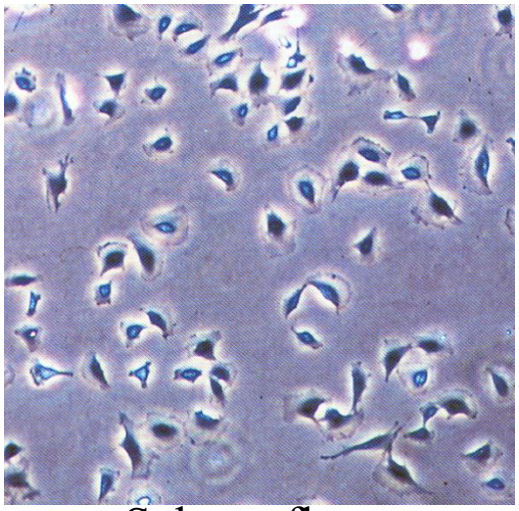
Phase contrast

Differential Interference Contrast (DIC) (beyond the scope here)

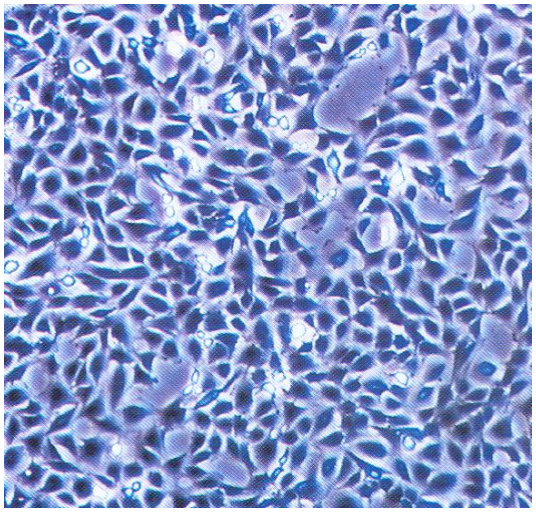


Growth and replating of cells in culture

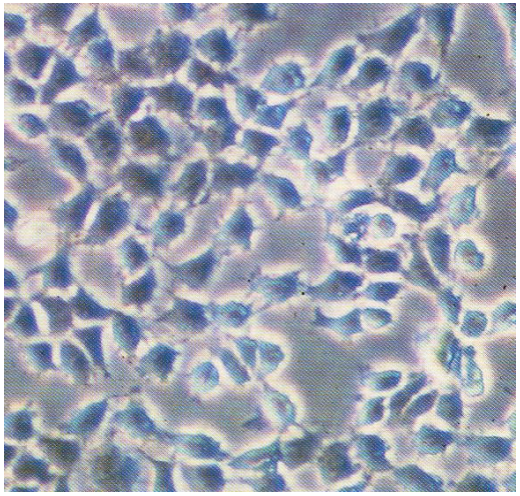




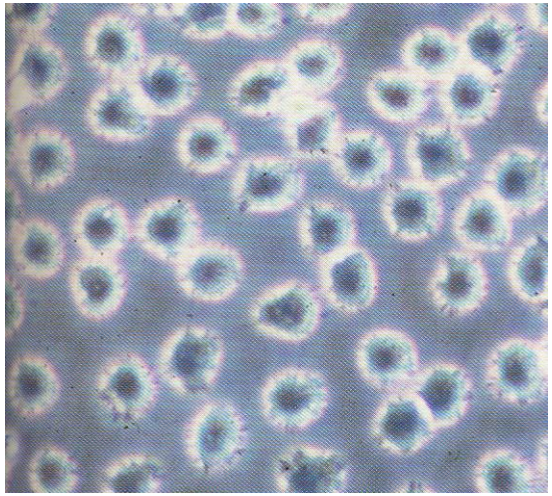
Sub-confluent,
exponential phase growth



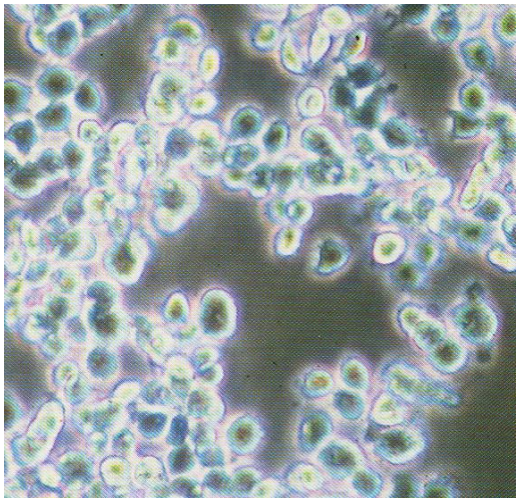
confluent monolayer



Passaging:
detach cells from surface

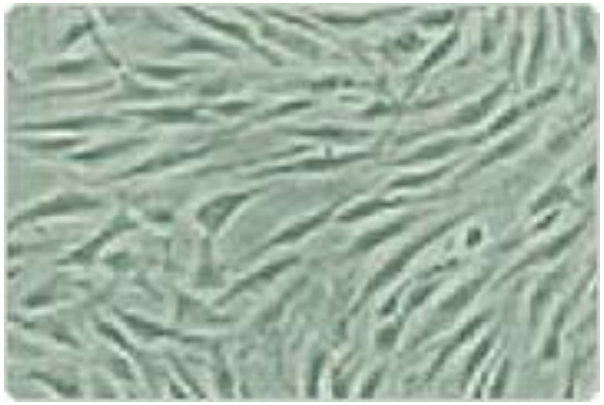


after trypsin removal

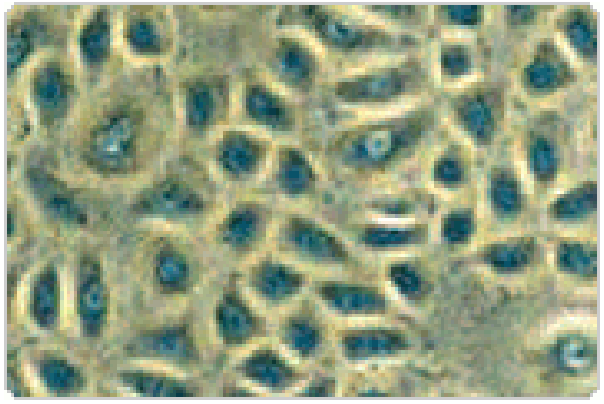


loosely attached cells
ready to split and replate

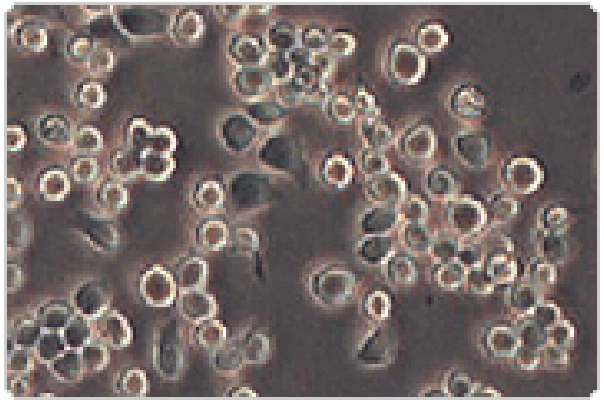
General classes of cell morphology



fibroblastic

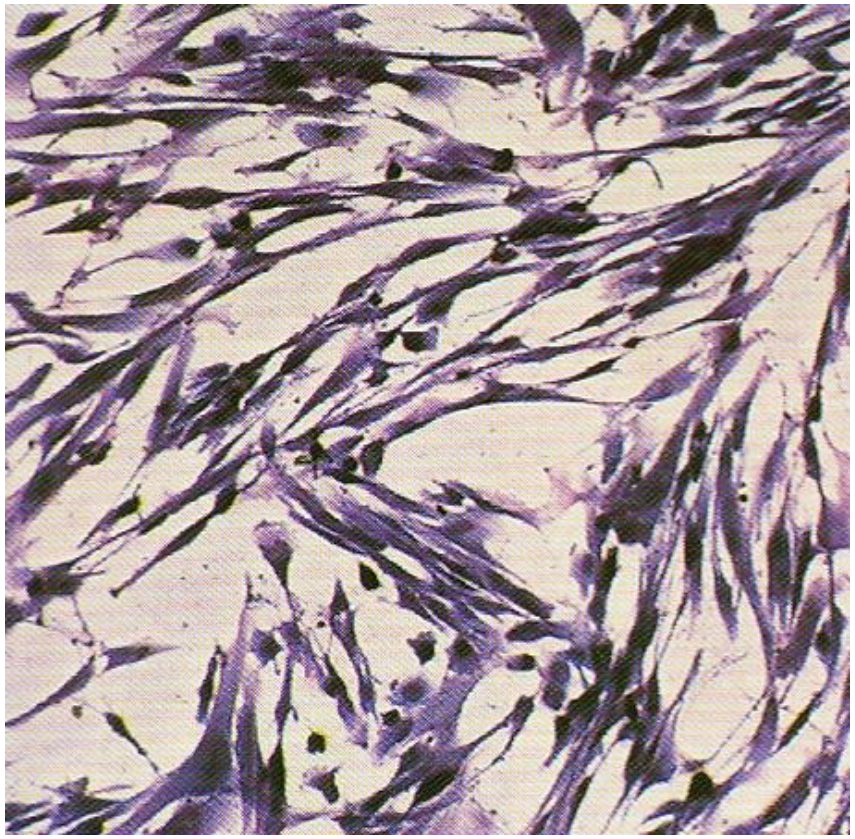


epithelial / cuboidal

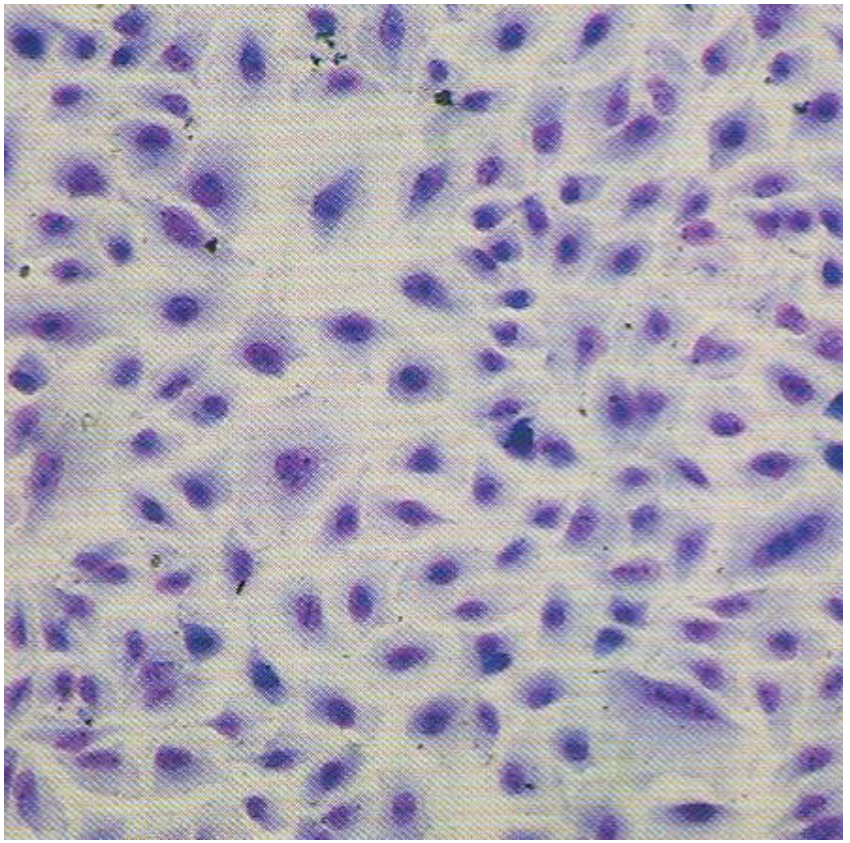


lymphoblastic

Primary cells vs. cell lines



Normal lung fibroblasts



lung adenocarinoma cells (A549)

Primary cells vs. cell lines

Primary cells

- Isolated from tissue and used before passaging.
- Closest to cells in the functional tissue
- Problems in defining a specific cell state phenotype, purity

Cell line

- Strict definition: any culture after first passaging
- Better defined cell populations, more uniform behavior.
- Selection for cells that grow in culture, less physiological.

Continuous cell line

- Cell line / subclone that has effectively unlimited proliferative potential.
- Can yield highly uniform, reproducible cell models that are easy to grow.
- Produced through natural or induced mutations
- Non-physiological behaviors.
- Well suited for studies of specific cellular / molecular processes, biologics factories, etc.

HeLa cell line as a model of cell growth

The HeLa cell line as a model of cell growth

- Henrietta Lacks died of cervical cancer at Johns Hopkins University Hospital (Oct. 4, 1951).
- Part of the cancer was broken down into individual cells and cultured. These cells showed extremely rapid and robust growth, became the first human cells to divide endlessly *in vitro*.
- Major impact on biological sciences and biotechnology.
- Major questions on how to ethically conduct research.

Cell lines, sources, and challenges

- Jurkat: model of T cells. From T cell leukemia patient.
- LRM55: astroglial cell line, from rat
- HEK293: Human Embryonic Kidney Cell Line 293
- MCF-7: Michigan Cancer Foundation, pleural effusion from 69-year old woman. Estrogen responsive.
- WI-38: normal human cell line, major applications in bitech. Lung tissue from legally aborted fetus
- iPSC: induced pluripotent stem cells