



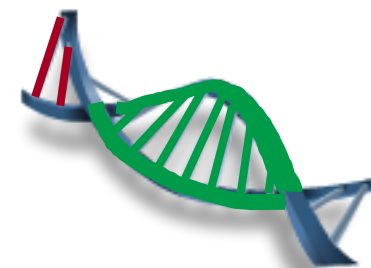
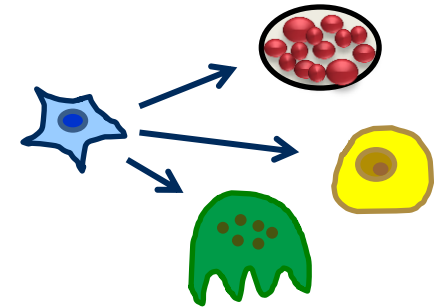
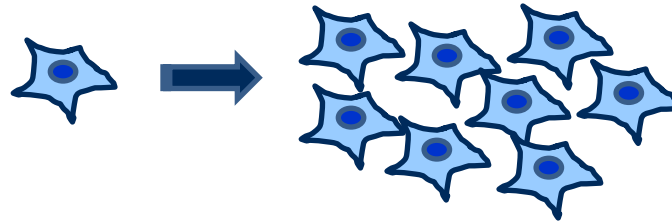
AME 50571

INTRODUCTION TO CELL CULTURE

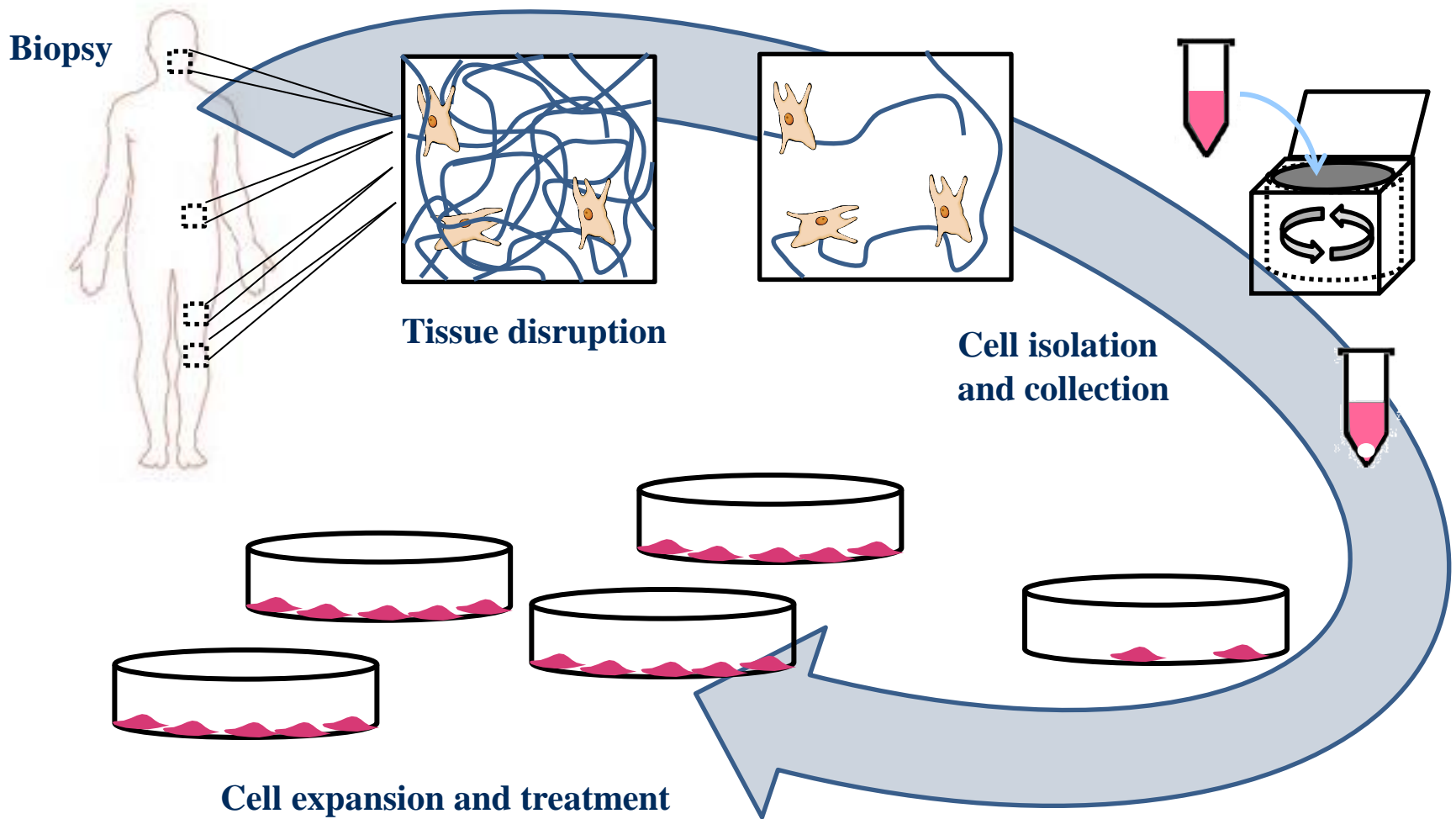


Why culture cells?

- Expansion
- Manipulation
 - Growth factors & cytokines
 - Genetic analysis
 - Investigation of molecular pathways
 - Analysis of cell behaviors
 - Induced Pluripotent Stem (iPS) cells
- Therapy
 - Cartilage repair
 - Stem cell treatments



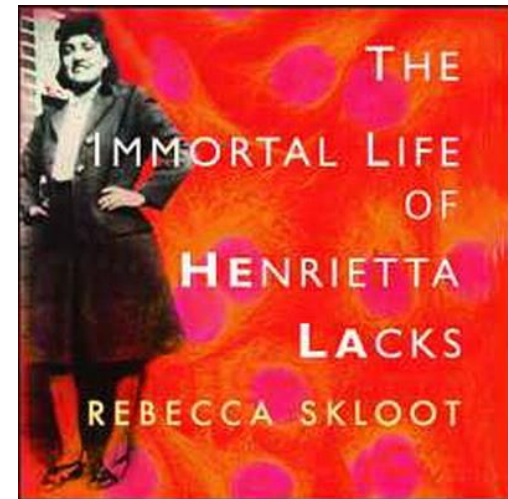
Cell Isolation



Cell Sources



- Primary cells
 - Isolated directly from an organism/tissue
 - Eg: bone marrow-derived stem cells, osteoblasts, chondrocytes, neurons, myocytes
- Cell lines
 - Cells derived from primary cells
 - Subcultures; subclones; select cells
 - Designed for sustained culture
 - Finite vs. Continuous/Immortal
 - Eg: HeLa



Cell 'playgrounds'

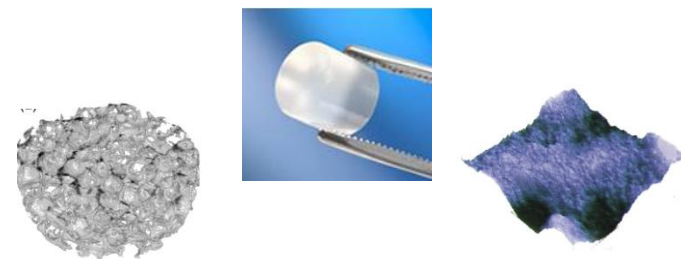
- 2-D

- Tissue culture plastic
 - Flasks, petri dishes



- 3-D

- Tissue engineering scaffolds
 - Solid: Titanium, polymers, natural materials and tissues
 - Soft: hydrogels, native tissues
 - Surfaces, networks, particles



- Bioreactor

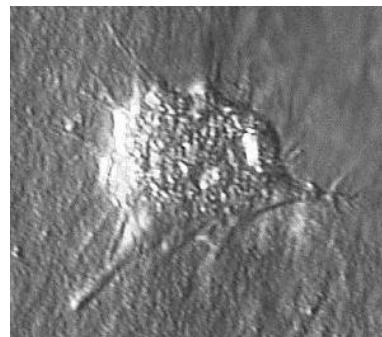
- 2-D or 3D with fluid flow

Caring for the cells



■ Environment

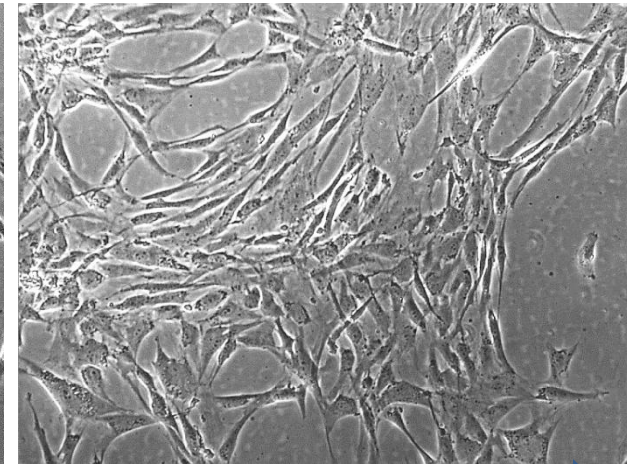
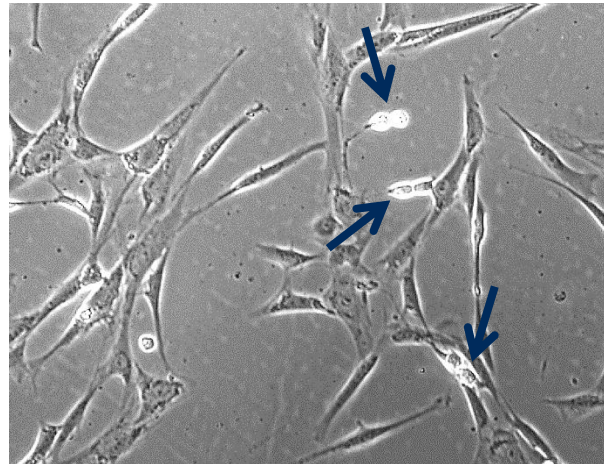
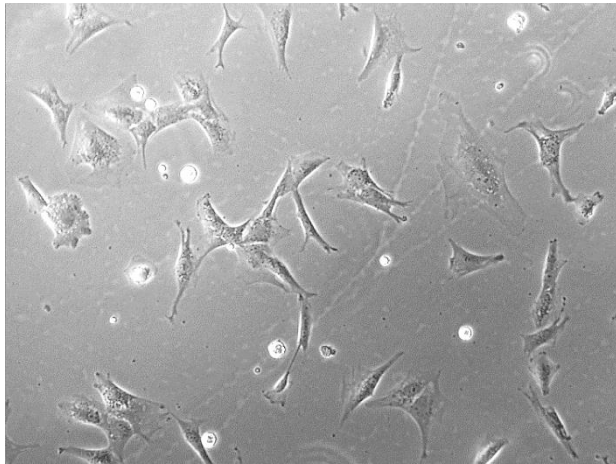
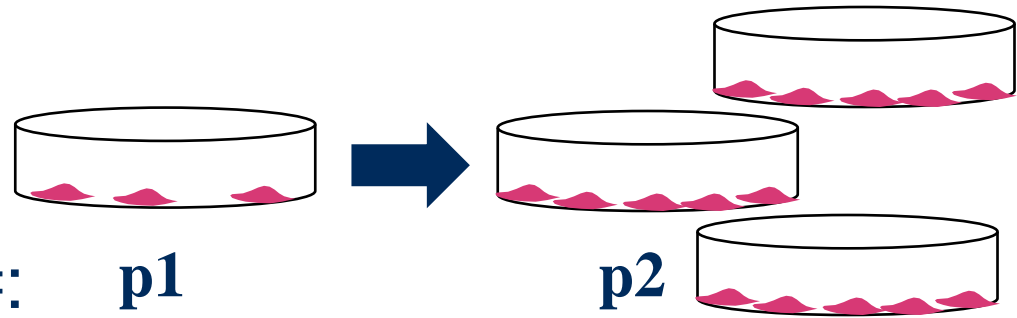
- Attachment
 - 2-D or 3-D surface
 - Growth area
- Nutrients
 - medium, eg: DMEM
- CO₂, humidity
- Temperature
 - 37 °C
- pH
 - 7.4



Expansion



- Increase cell number
 - ‘Passaging’
 - ‘Splitting’
 - Designated by #: p1

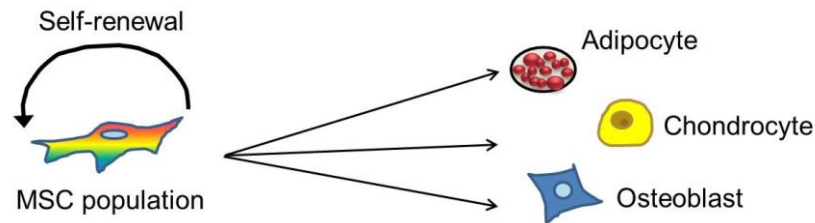


Confluence

Differentiation



- Differentiate precursor cells with appropriate combination of cues

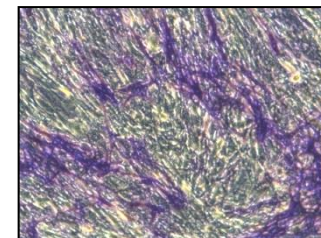
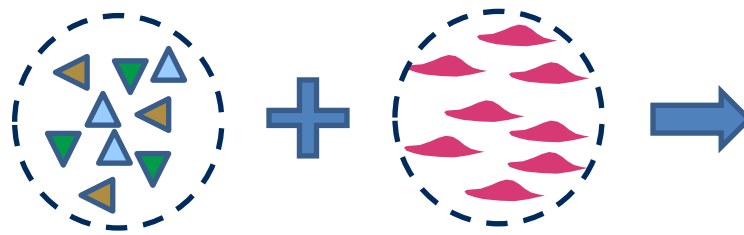
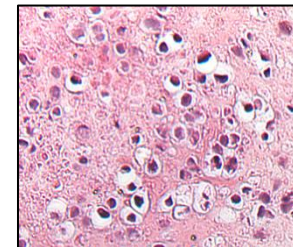


- Chemical

- eg: Ascorbic acid, β -glycerophosphate, dexamethasone, Bone Morphogenetic Proteins

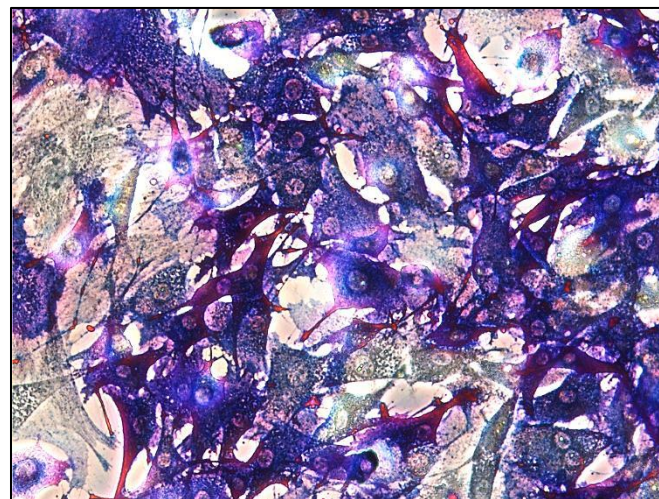
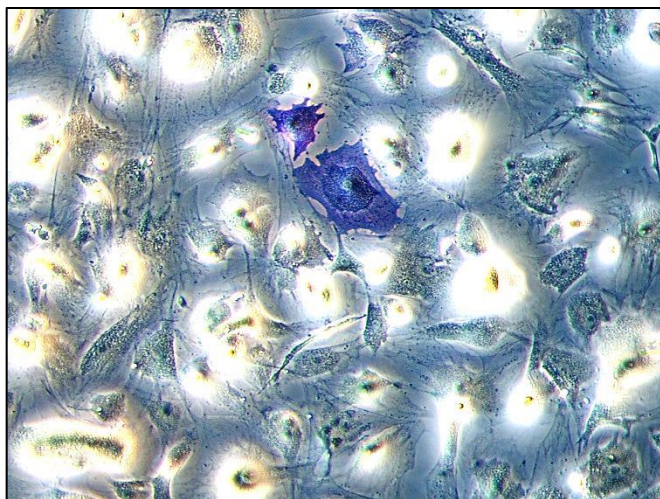
- Mechanical

- Eg: scaffold properties; applied loading



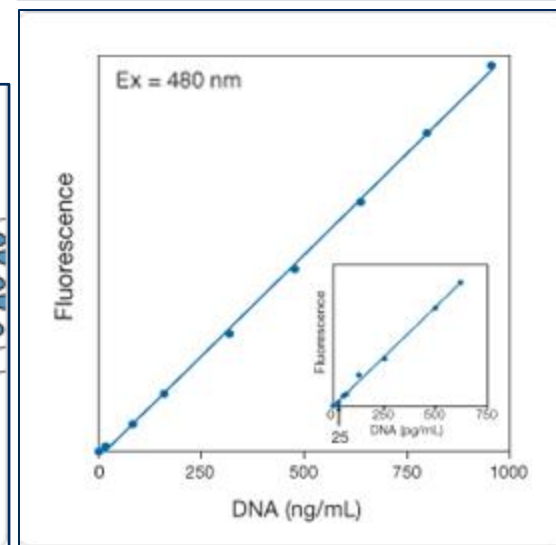
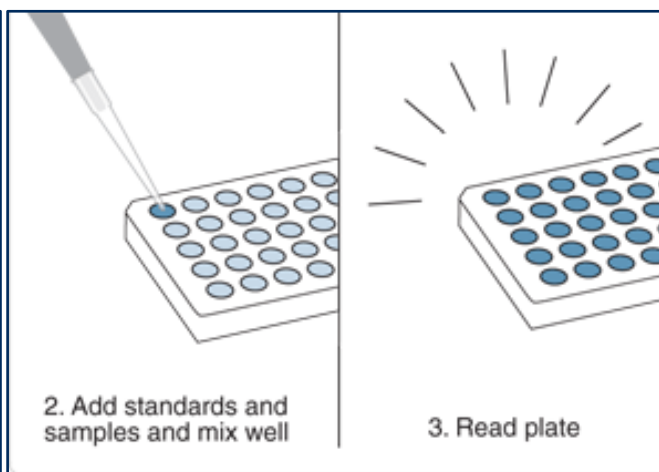
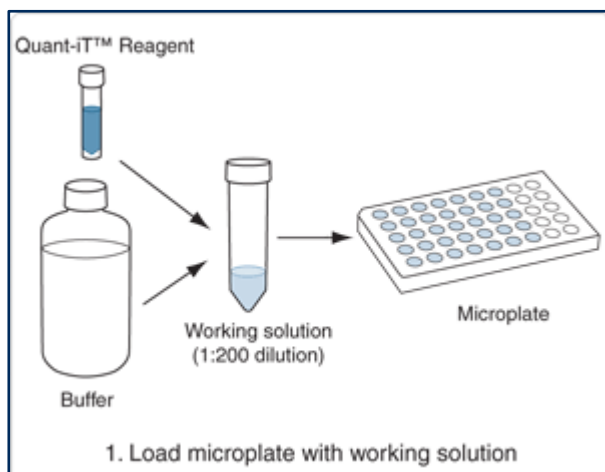
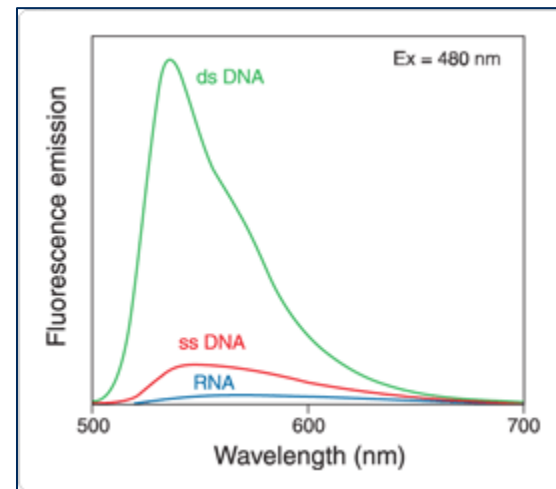
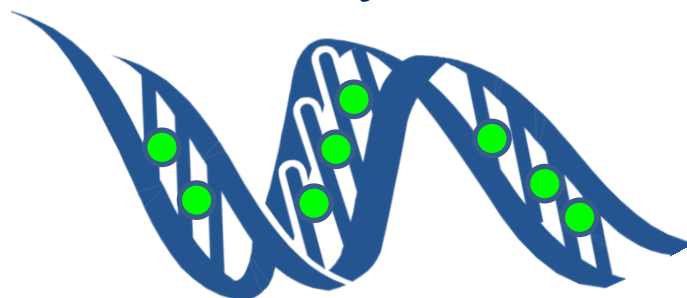
Analysis: ALP Activity

- Alkaline phosphatase (ALP)
 - Enzyme that cleaves phosphate groups of organic phosphates
 - Releases inorganic phosphates required for mineralization



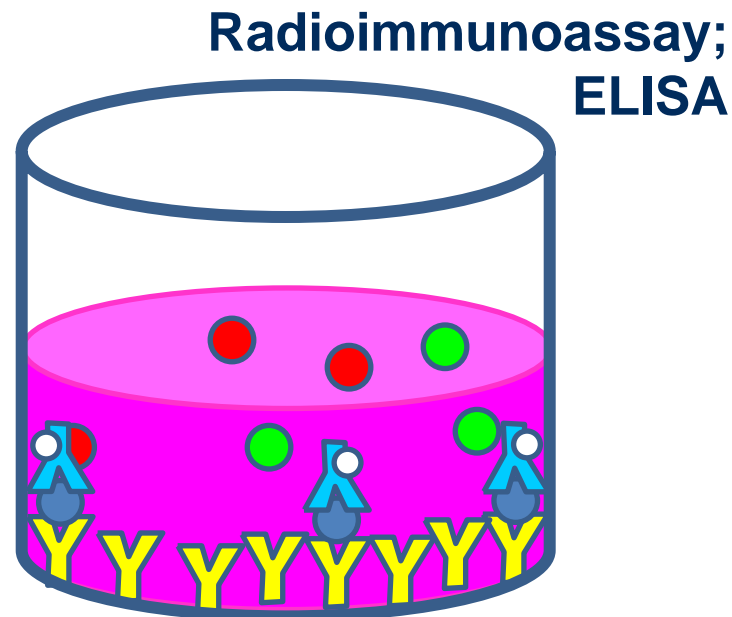
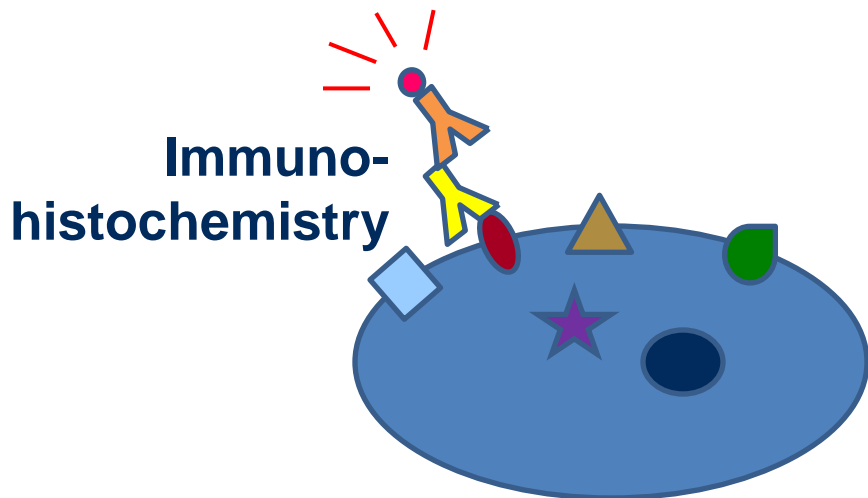
Analysis: DNA Content

- DNA Quantification
 - PicoGreen assay



Analysis: Immunoassays

- Targets *antigens* in samples with labeled *antibodies*
 - Visualized by tags or enzymes
 - Fluorescent
 - Radioactive

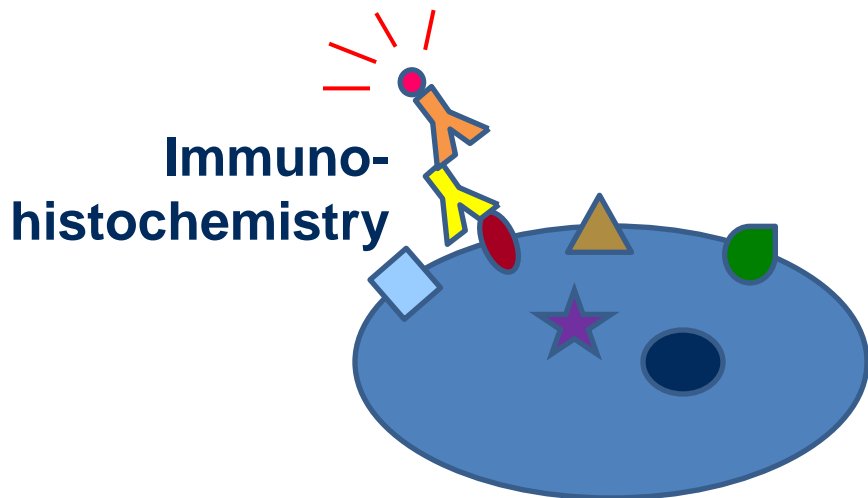


Analysis: Immunoassays

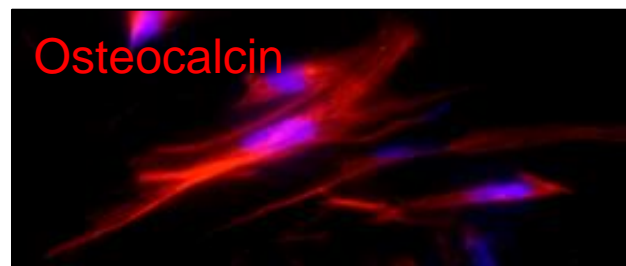
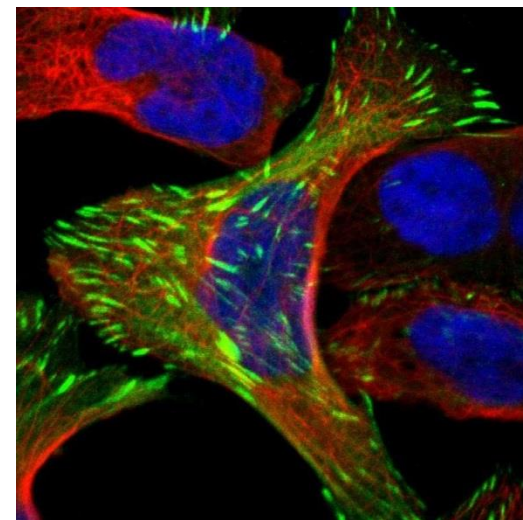
- Targets *antigens* in samples with labeled *antibodies*

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Actin
Nucleus
Focal
Adhesion



Questions?

