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Advancing ideas.

Human Cell Analysis

The Technology Behind The World's Most Common Diagnostic Test

IEEE-CNSV – November 4, 2014

Giacomo Vacca, Ph.D.
Founder & President
Kinetic River Corp.

OVERVIEW

- Blood
- Components of blood
- Blood diagnostics
- Counting blood cells
- Principles of flow cytometry
- Flow cytometry systems
- Flow cytometry system architecture
- Developing flow cytometers

If there's time…

- Flow cytometry data analysis
- Disruptive innovations

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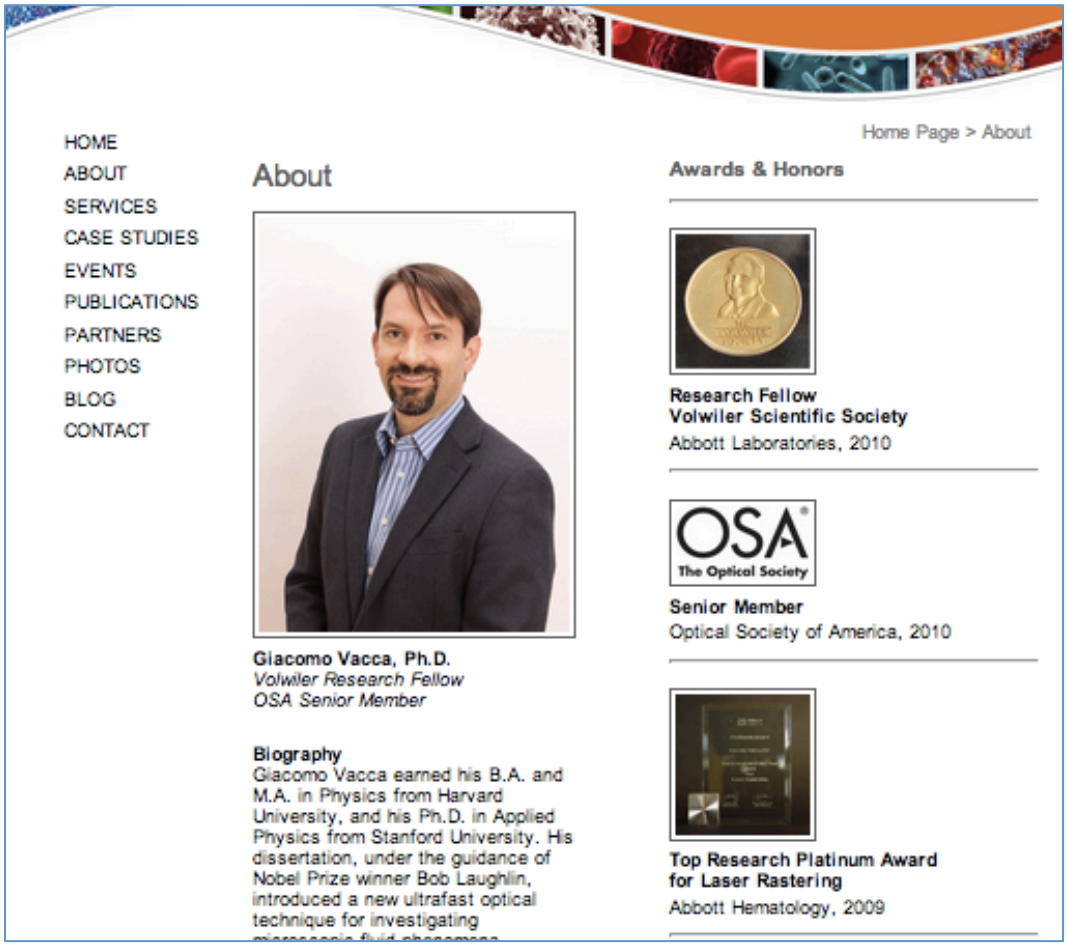

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About

Giacomo Vacca, Ph.D.
Volwiler Research Fellow
OSA Senior Member

Biography
Giacomo Vacca earned his B.A. and M.A. in Physics from Harvard University, and his Ph.D. in Applied Physics from Stanford University. His dissertation, under the guidance of Nobel Prize winner Bob Laughlin, introduced a new ultrafast optical technique for investigating

Awards & Honors

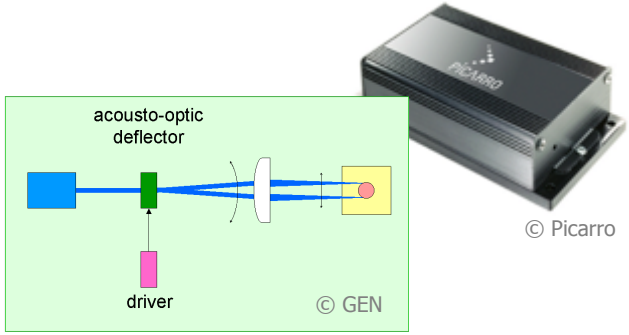
Research Fellow
Volwiler Scientific Society
Abbott Laboratories, 2010

OSA The Optical Society

Senior Member
Optical Society of America, 2010

Top Research Platinum Award for Laser Rastering
Abbott Hematology, 2009

- **Kinetic River** (President)
- **BeamWise** (CSO)
- Abbott Labs (Res. Fellow)
- Picarro
- Lightwave Micro
- Exxon Research & Engr.
- 28 patents issued/pending



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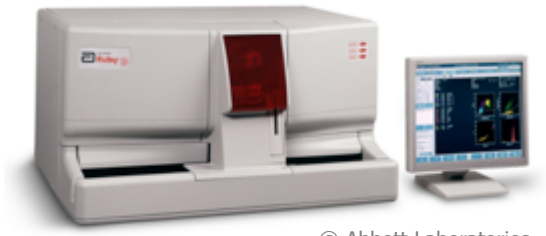

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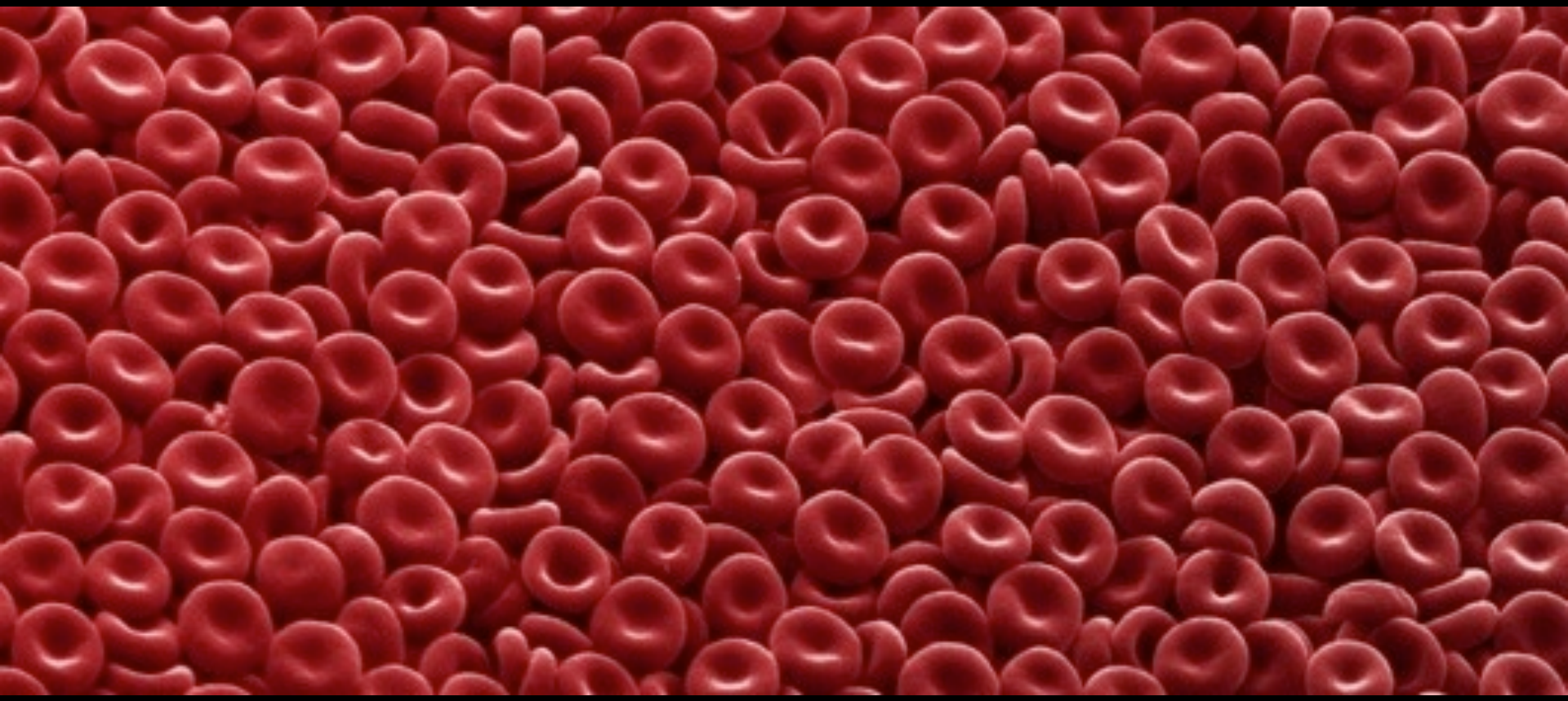
BLOOD



What Is It About Blood?

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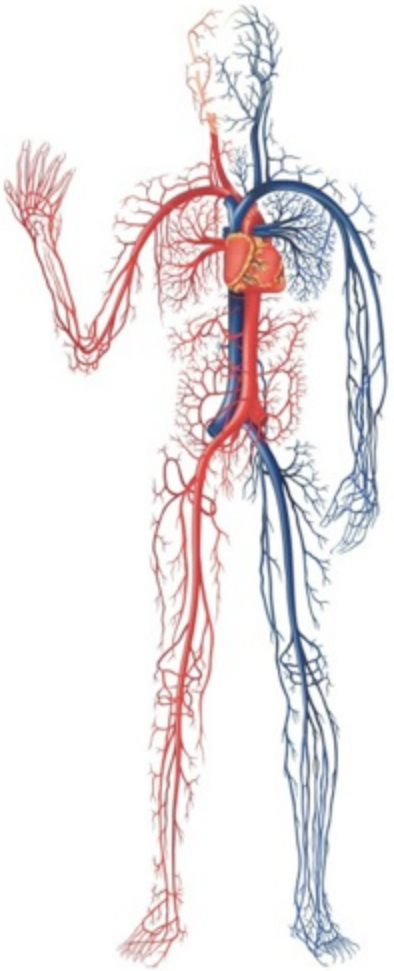
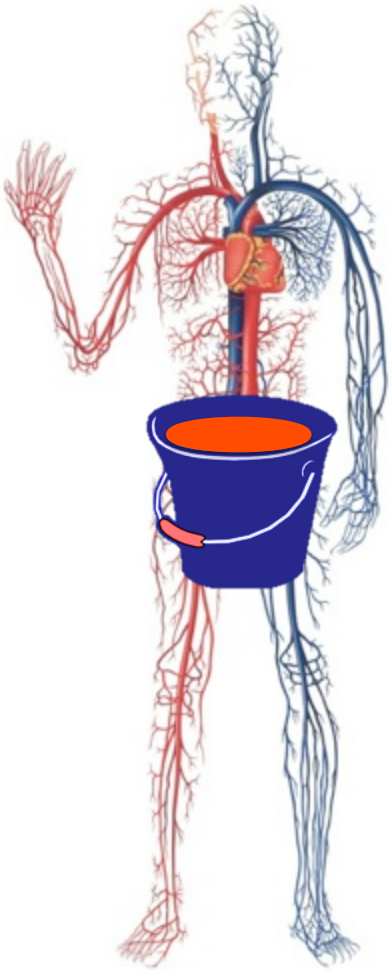
Blood



6 Quarts



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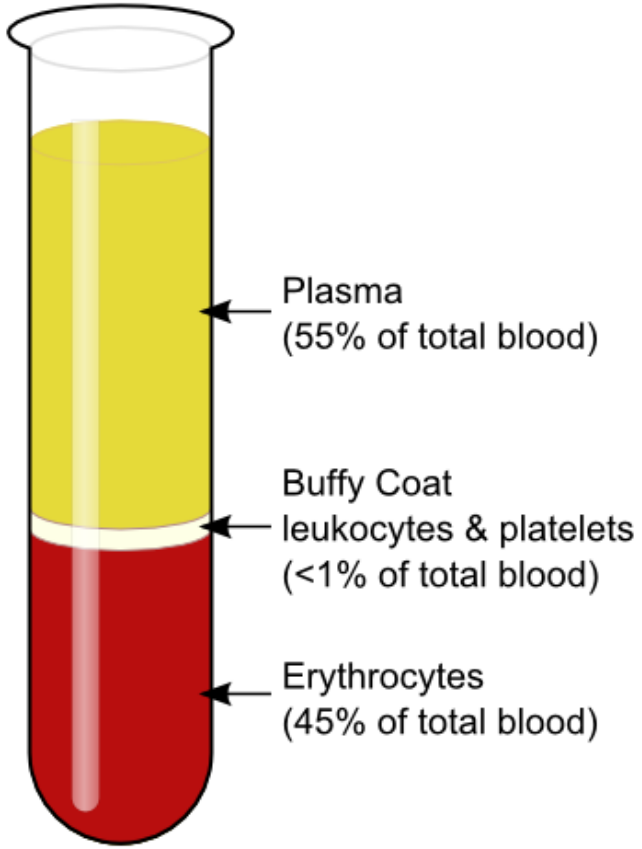
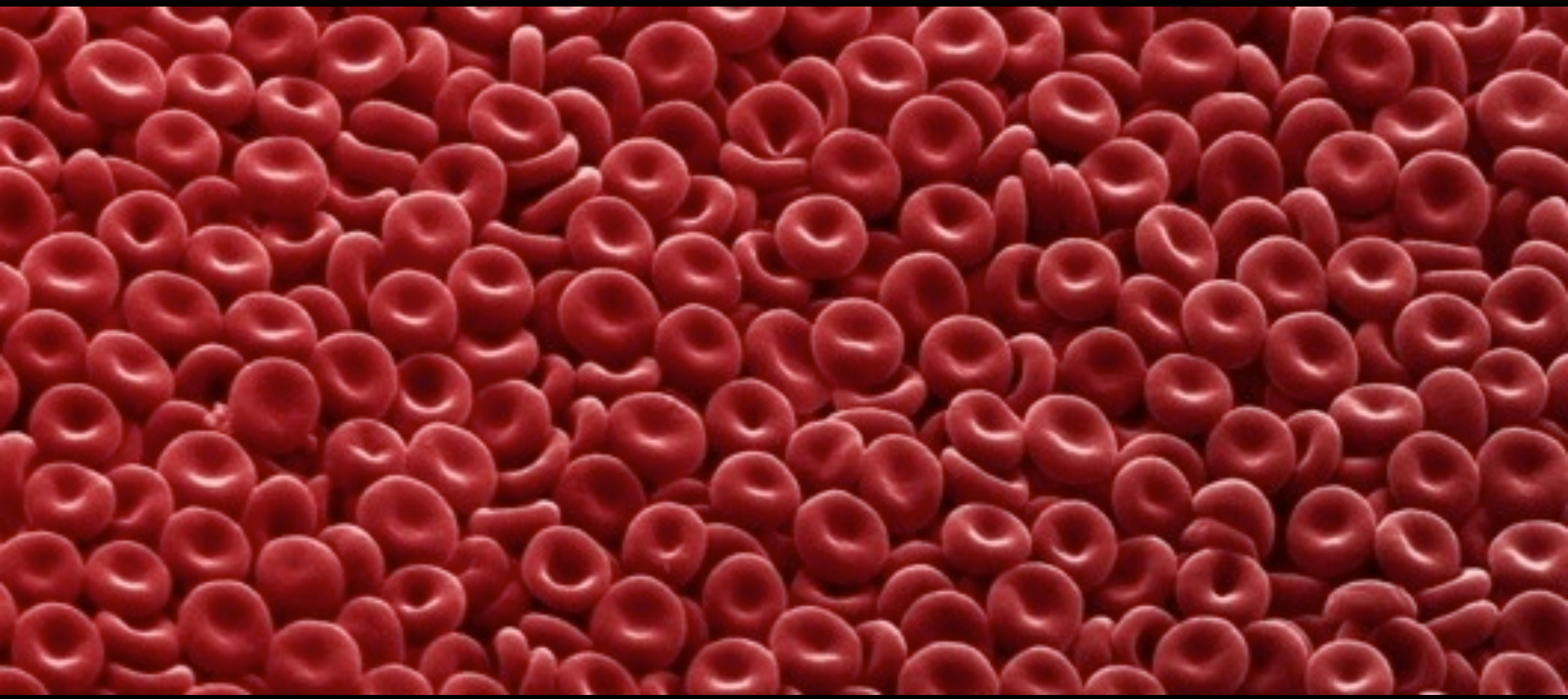
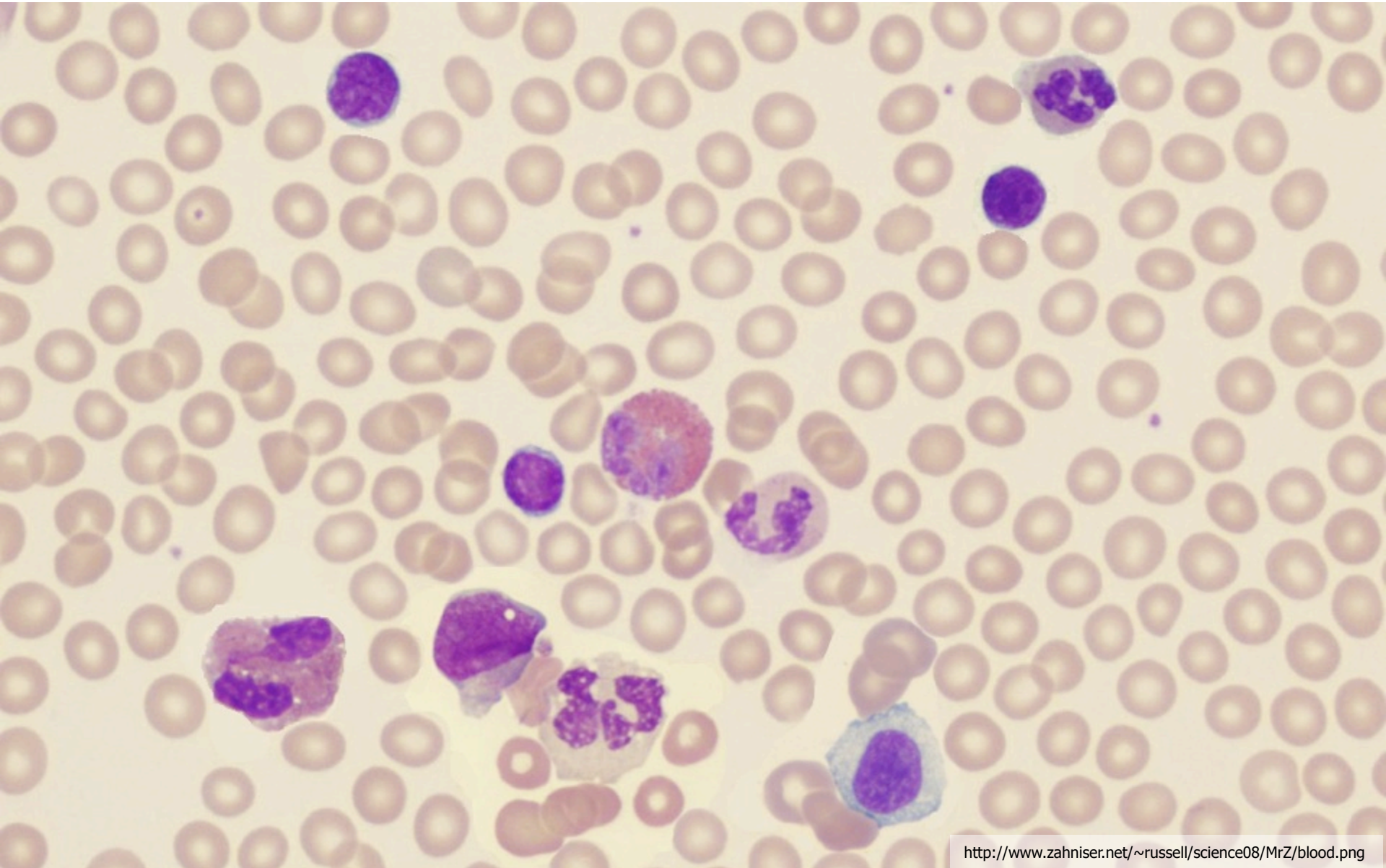
It’s In Your Blood…



COMPONENTS OF BLOOD

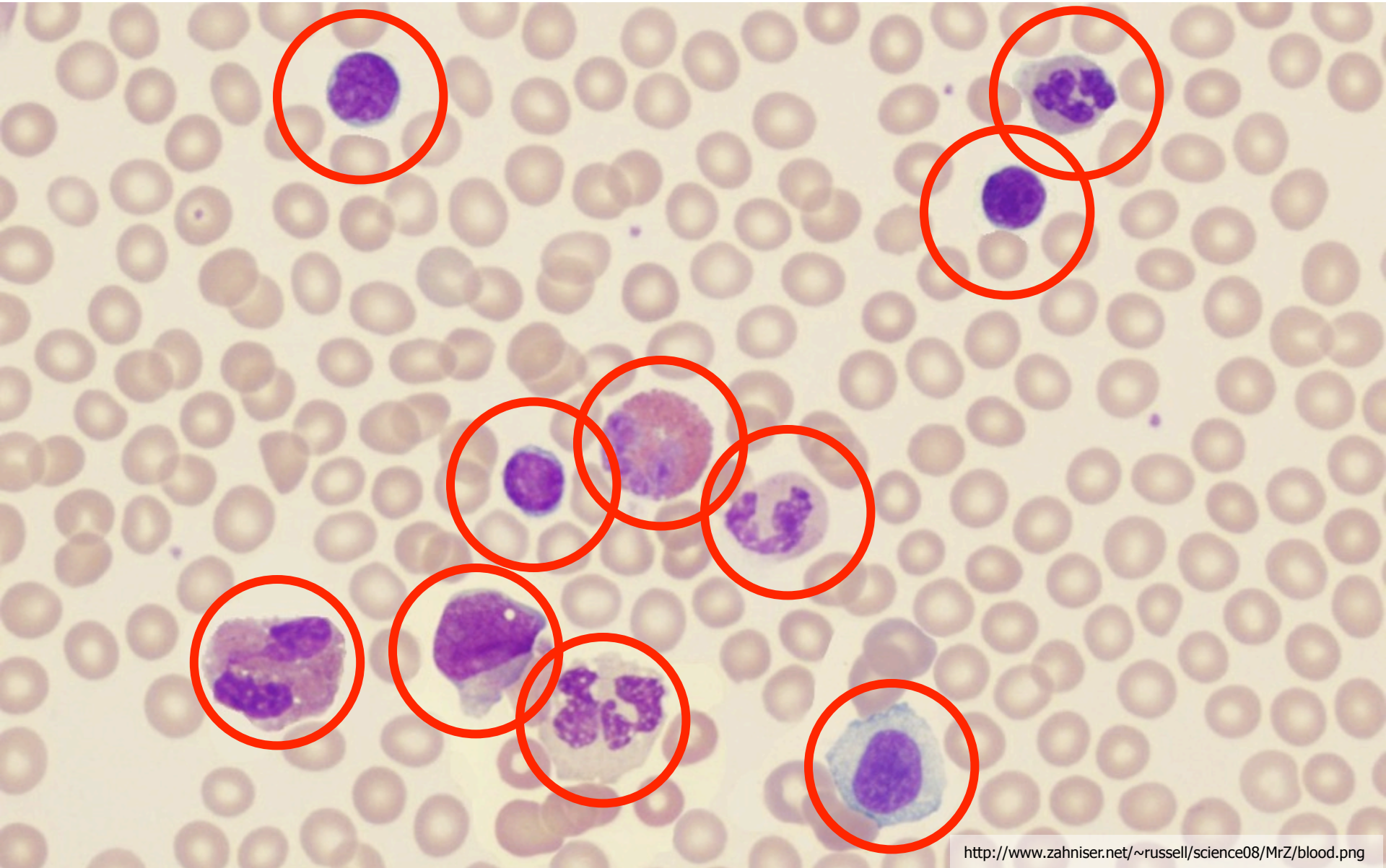


White Blood Cells (WBCs)



http://www.zahniser.net/~russell/science08/MrZ/blood.png

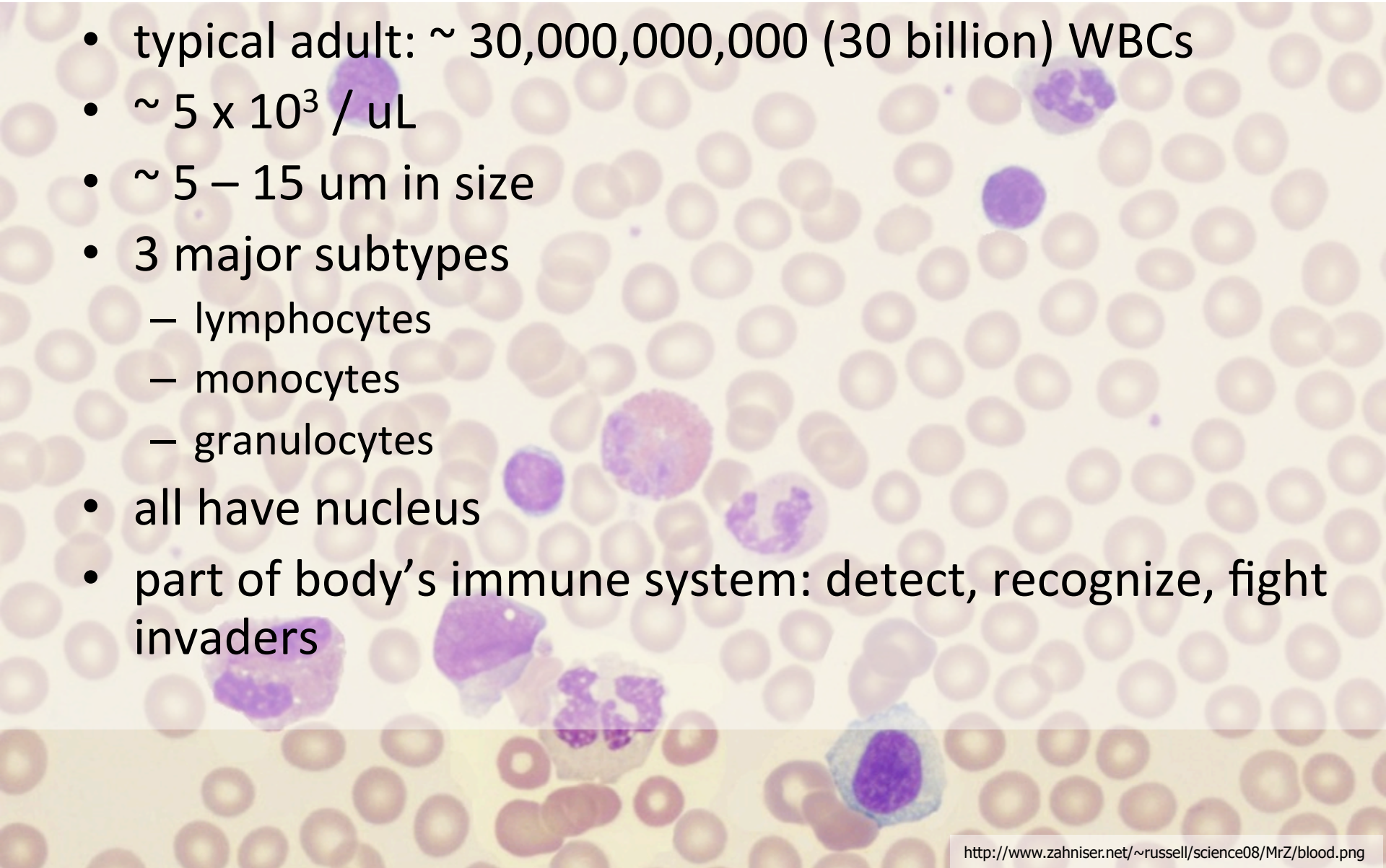
WBCs (*aka* Leukocytes)



http://www.zahniser.net/~russell/science08/MrZ/blood.png

WBC Facts

- typical adult: ~ 30,000,000,000 (30 billion) WBCs
- ~ 5 x 10^3 / uL
- ~ 5 – 15 um in size
- 3 major subtypes
  - lymphocytes
  - monocytes
  - granulocytes
- all have nucleus
- part of body's immune system: detect, recognize, fight invaders



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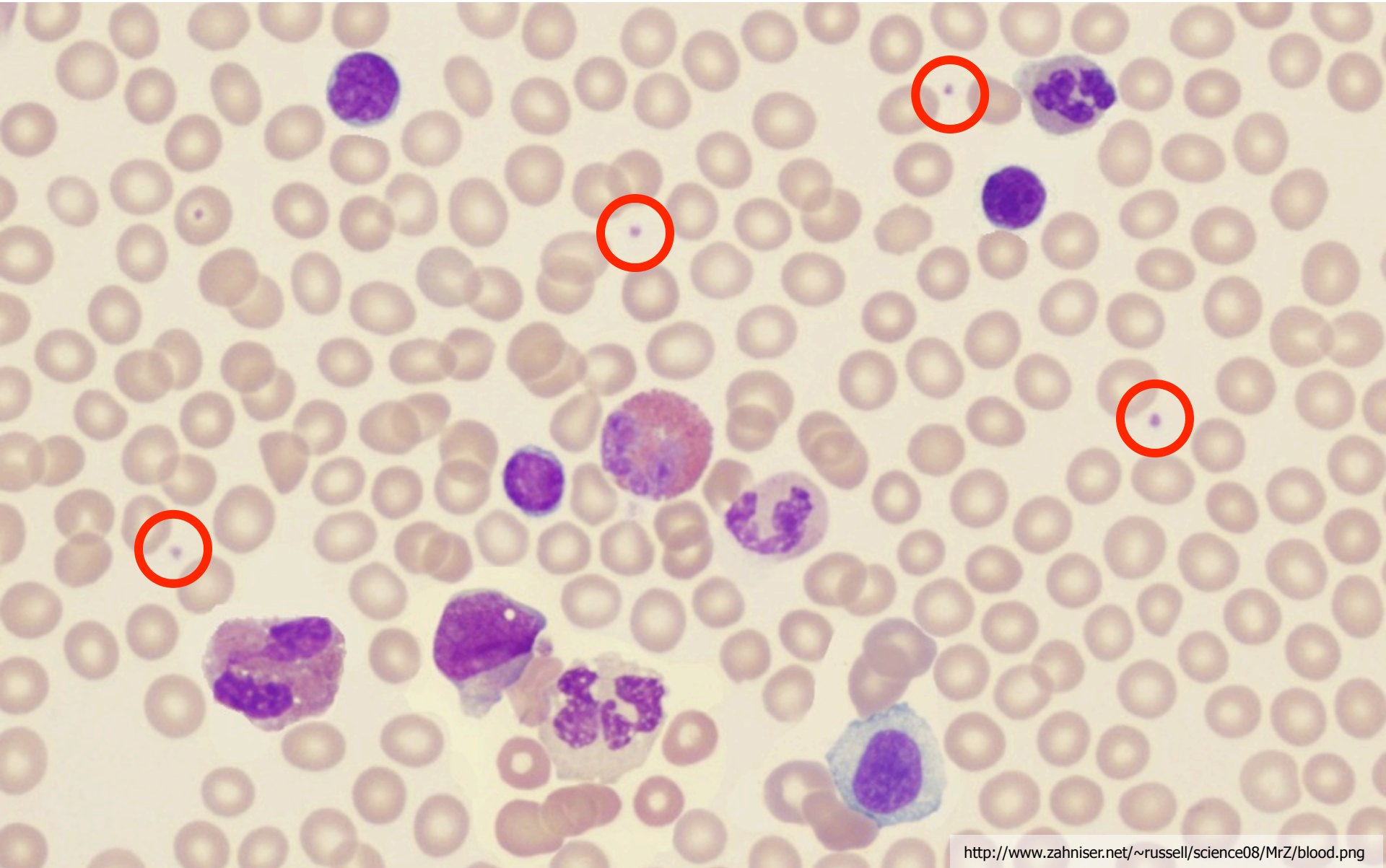
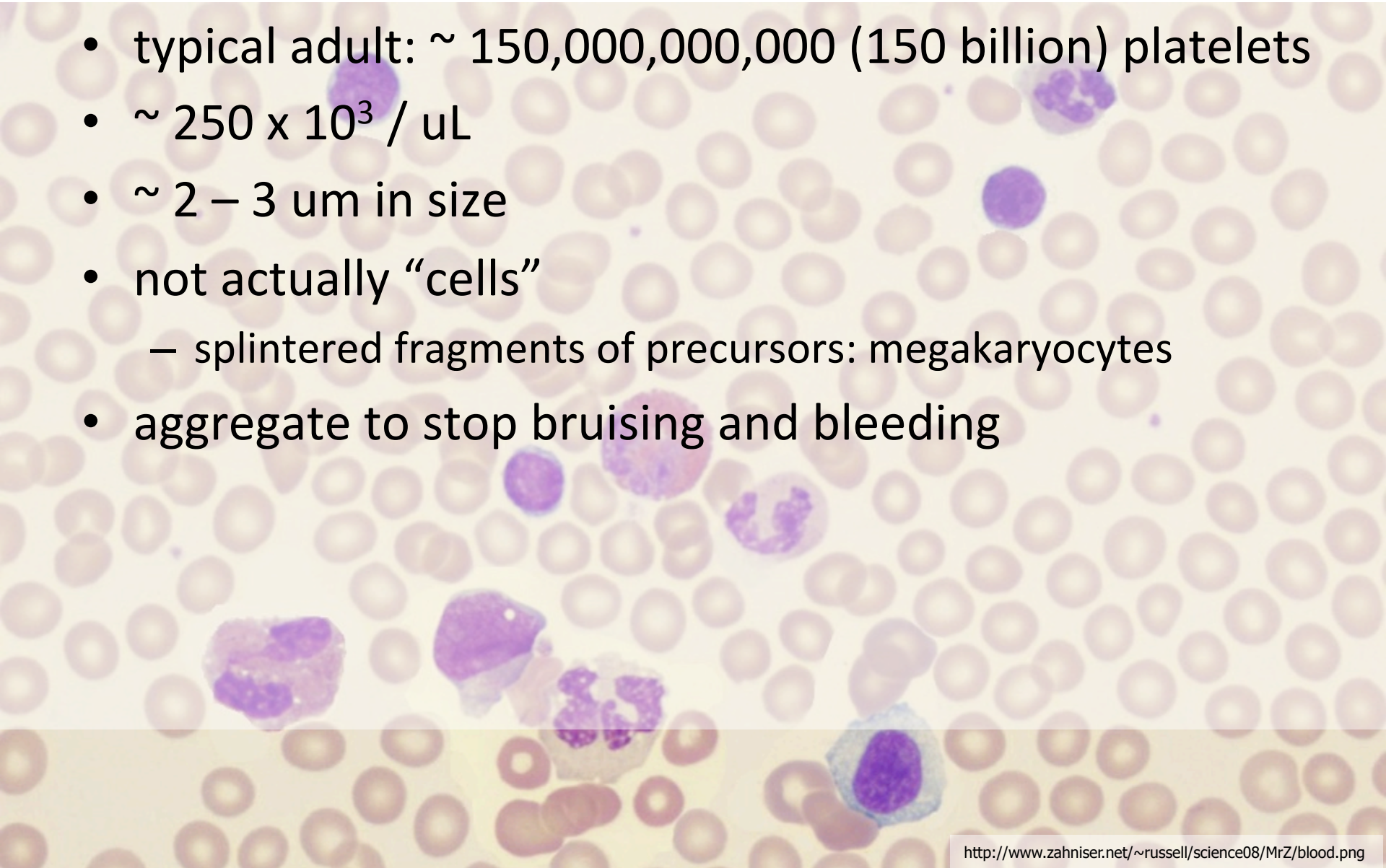
Platelets (*aka* Thrombocytes)



http://www.zahniser.net/~russell/science08/MrZ/blood.png

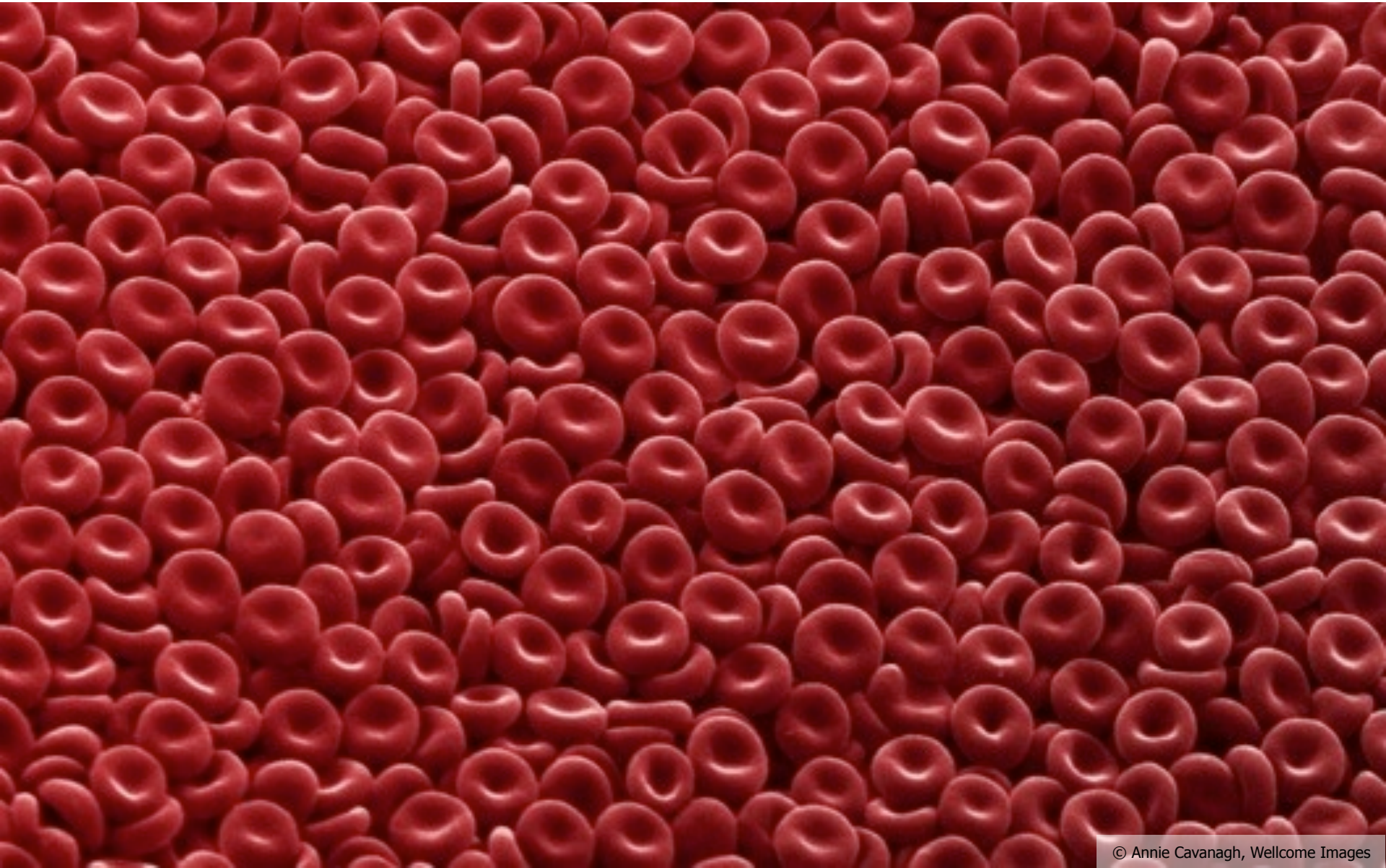
Platelet Facts

- typical adult: ~ 150,000,000,000 (150 billion) platelets
- ~ 250 x 10^3 / uL
- ~ 2 – 3 um in size
- not actually “cells”
  - splintered fragments of precursors: megakaryocytes
- aggregate to stop bruising and bleeding

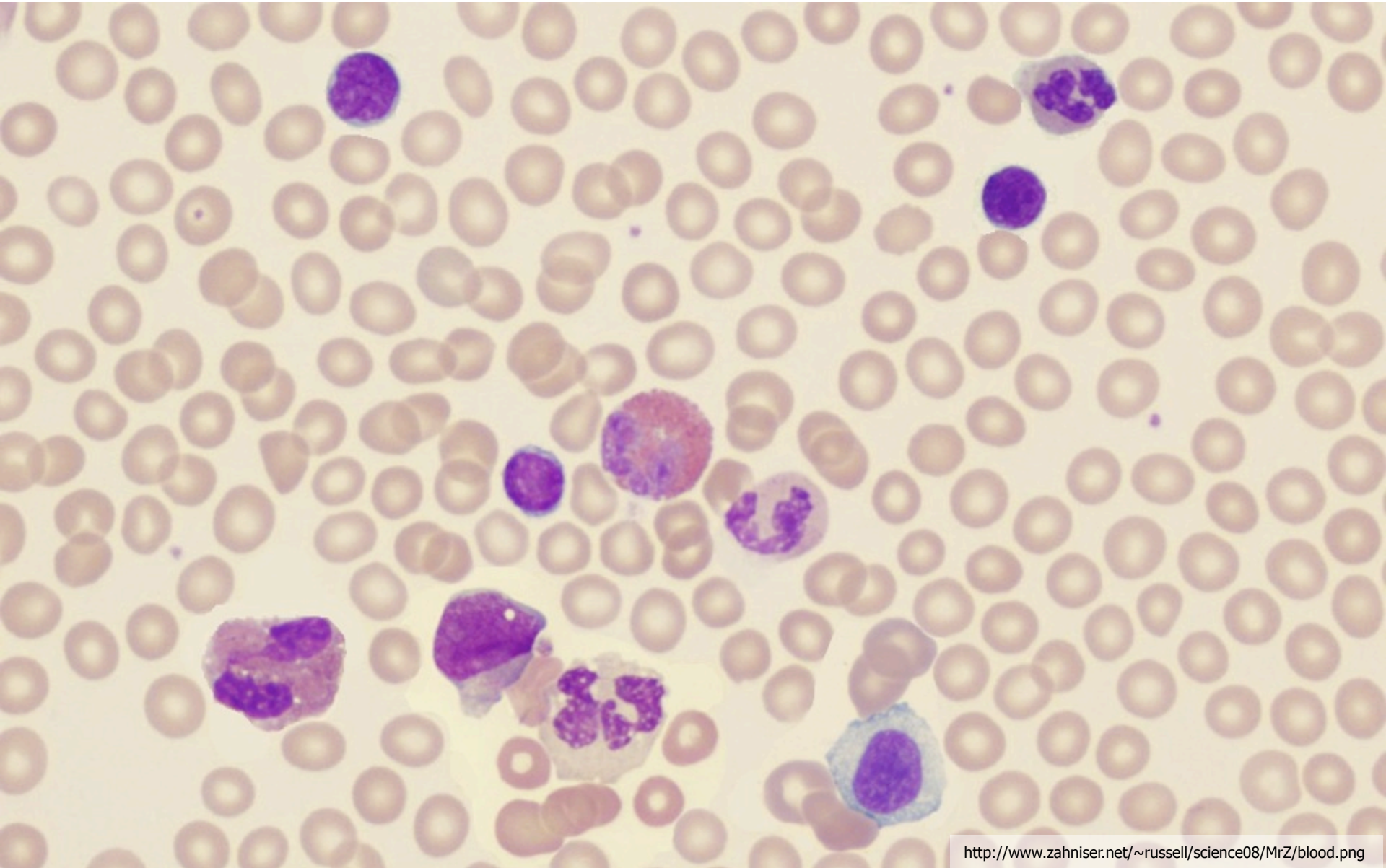


http://www.zahniser.net/~russell/science08/MrZ/blood.png

Red Blood Cells (RBCs)



RBCs (*aka* Erythrocytes)



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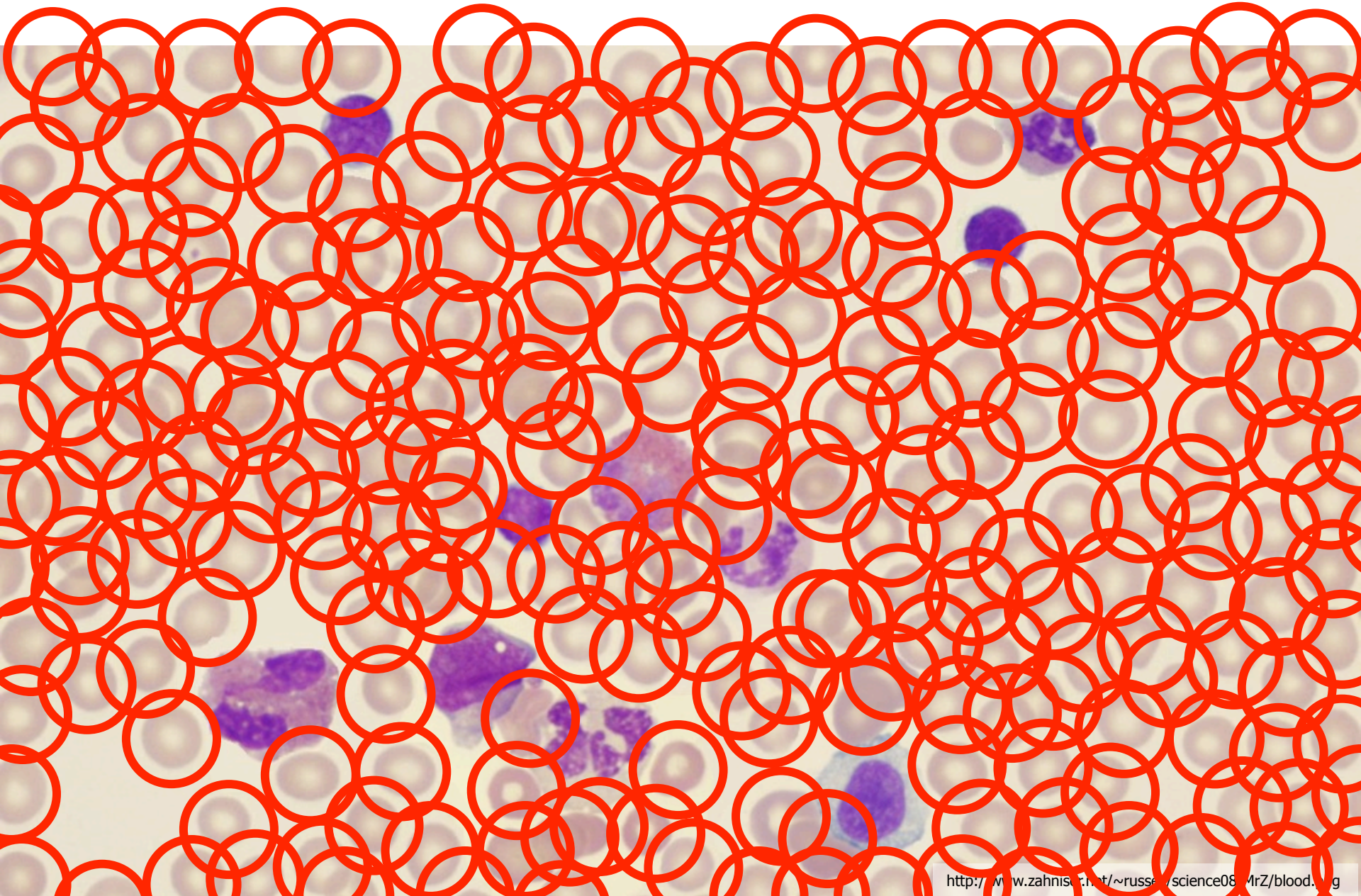
RBCs

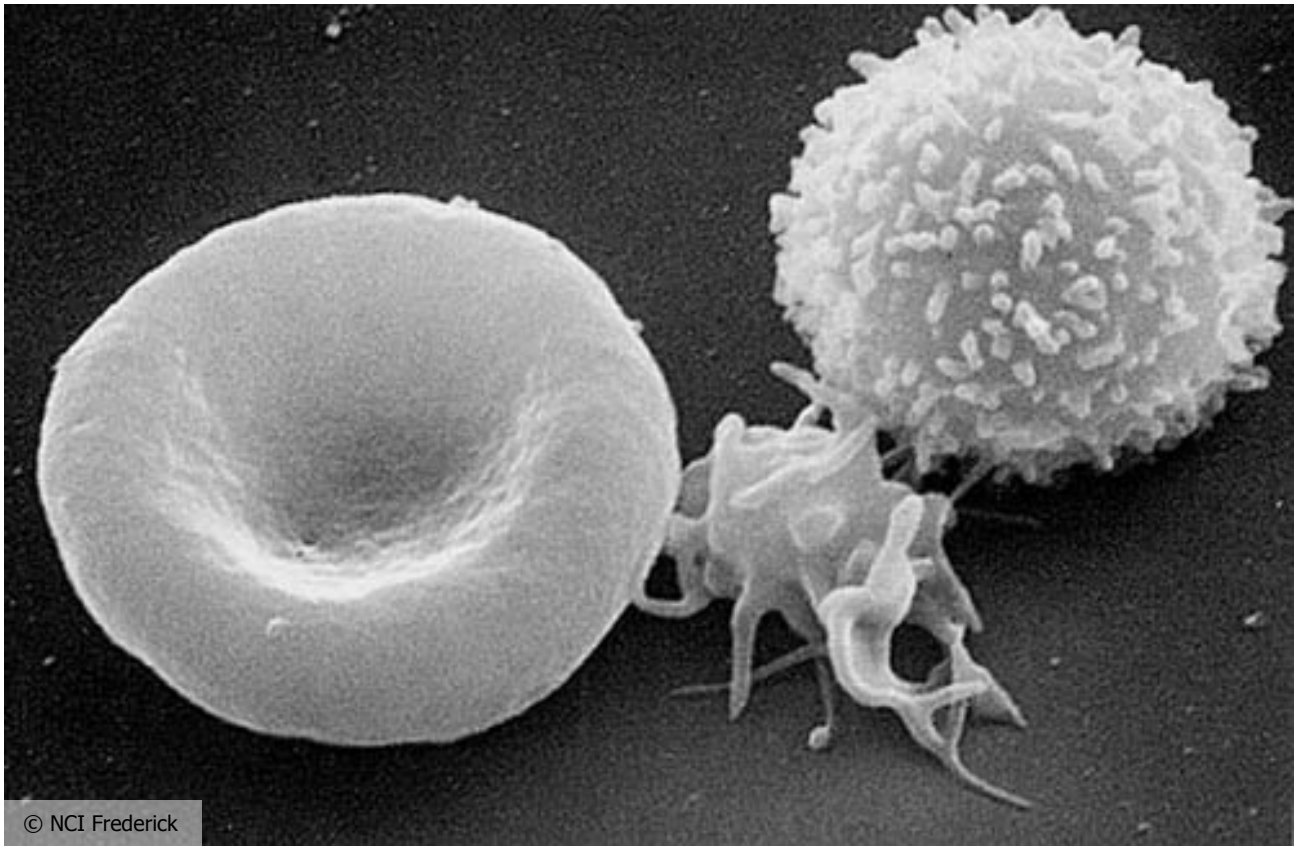


RBC Facts

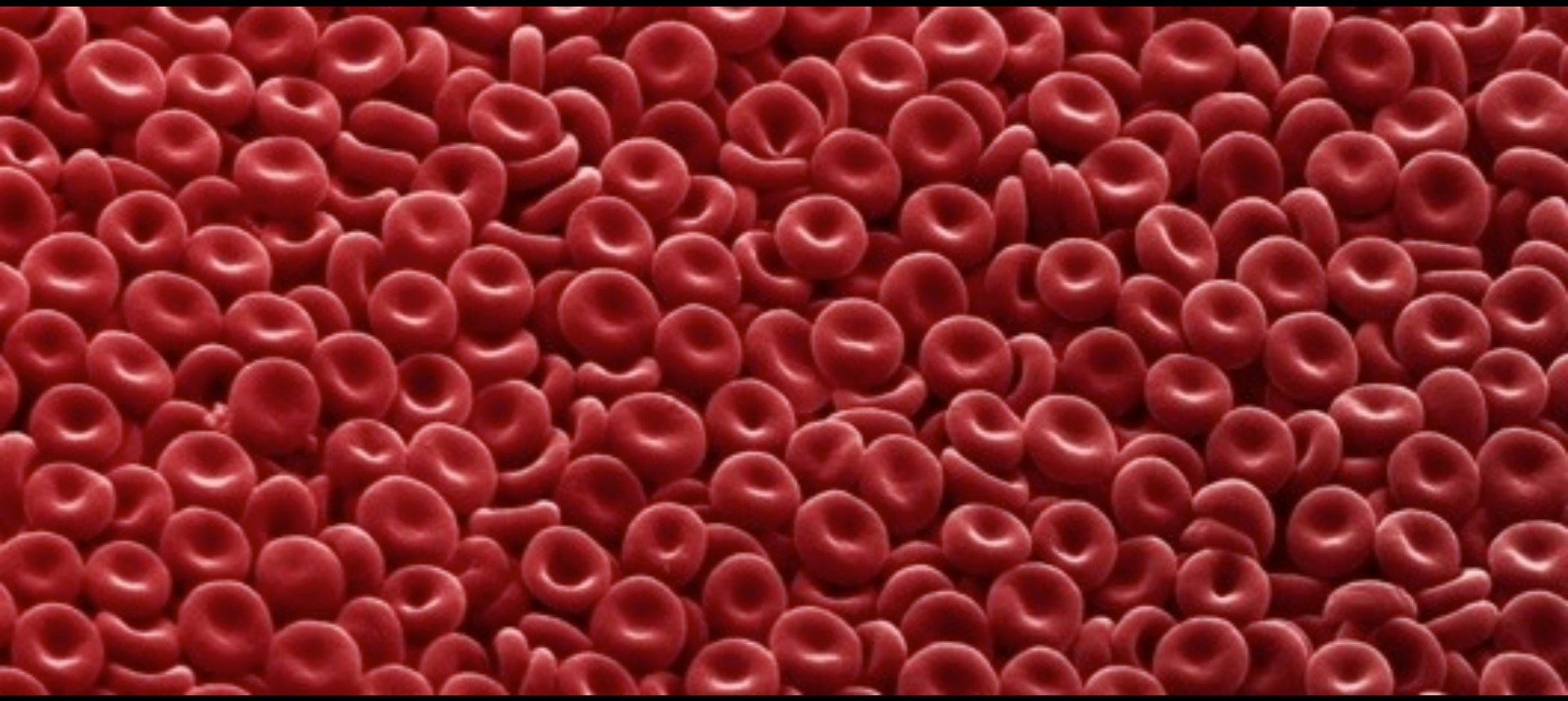
- typical adult: ~ 30,000,000,000,000 (30 trillion) RBCs
- 3 million RBCs produced per second
- 120 days lifetime
- ~ 5×10^6 / uL
- ~ 45% of blood by volume
- ~ 5 um in size
- characteristic biconcave shape
- no nucleus! “hemoglobin bags”
- carry O_2 from lungs to tissues, carry back CO_2

http://www.zahniser.net/~russell/science08/MrZ/blood.png

The Three Amigos



BLOOD DIAGNOSTICS



Blood-Based Diagnostics

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Blood-Based Laboratory Diagnostics

- Cell assays
  - Complete Blood Count (CBC)
  - hematocrit
  - hemoglobin
  - WBC differential
  - platelet count
  - immature RBCs
  - …
- Plasma assays
  - glucose
  - electrolytes
  - lipids
  - blood gases
  - proteins
  - enzymes
  - …
- Other diagnostic assays
  - molecular diagnostics
  - blood typing
  - DNA sequencing
  - …

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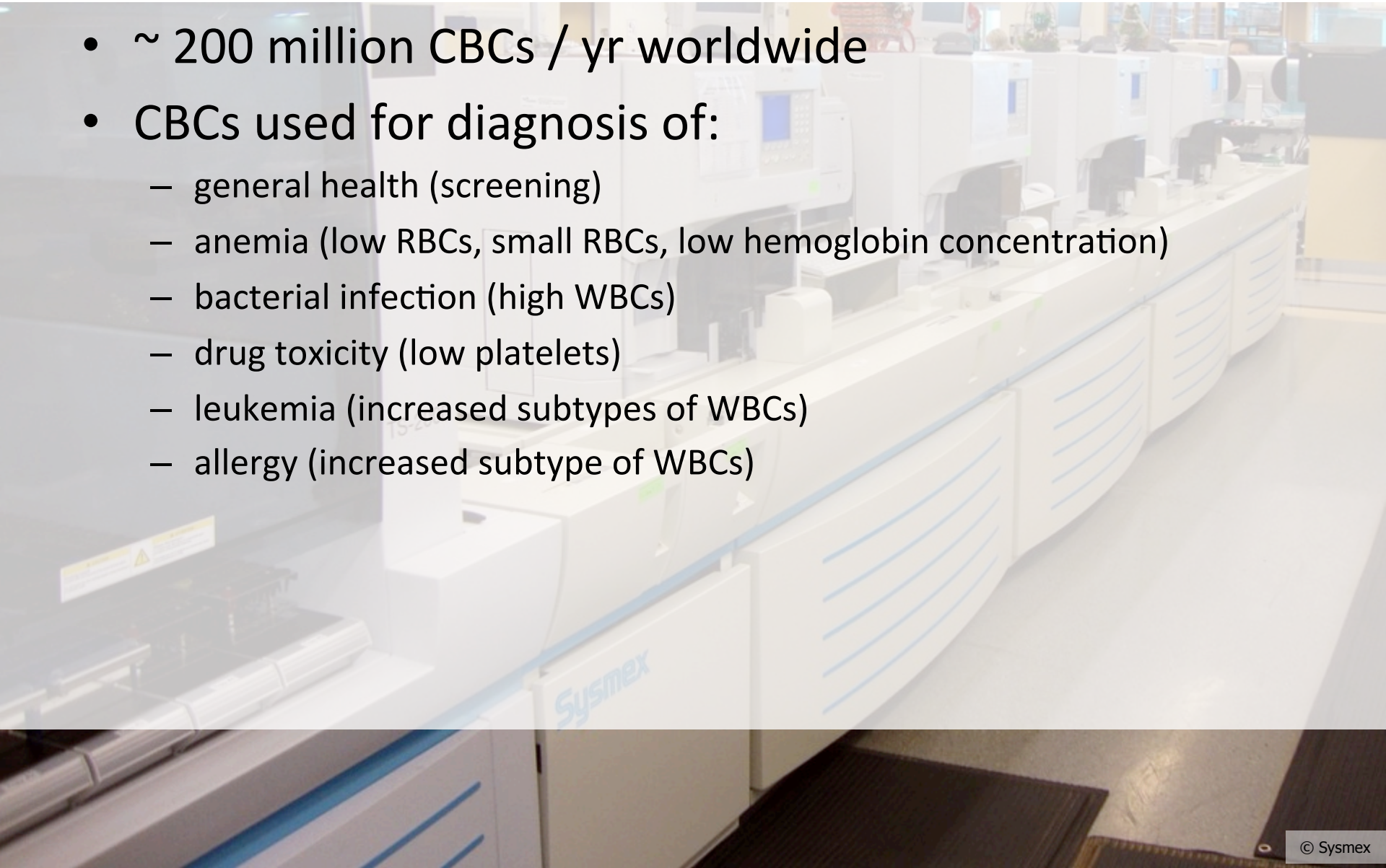
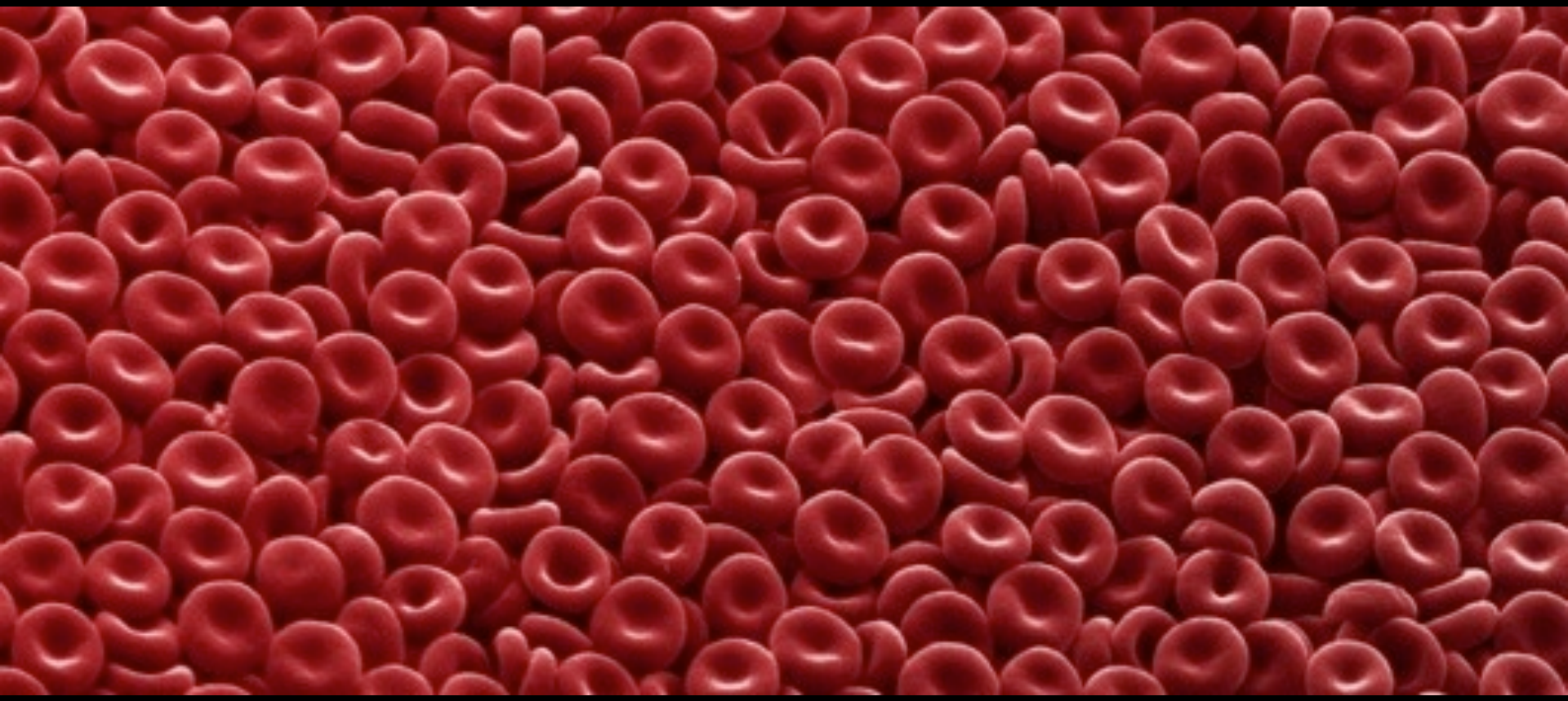
Complete Blood Count (CBC)

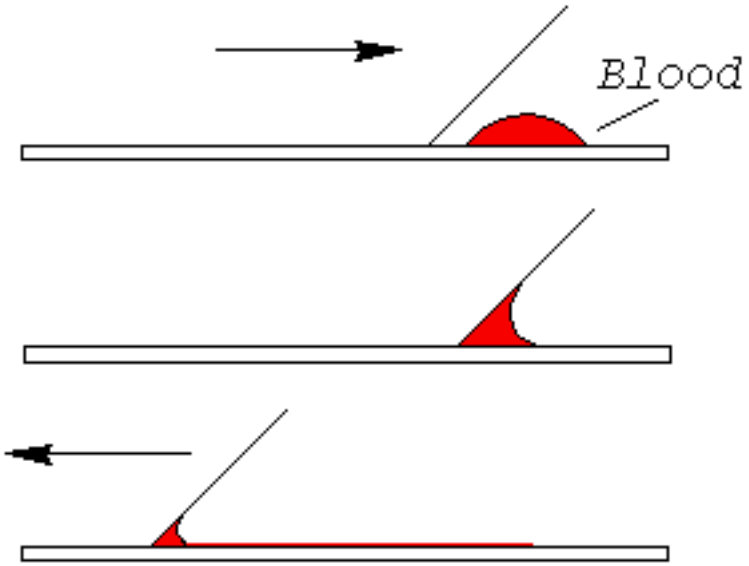
- ~ 200 million CBCs / yr worldwide
- CBCs used for diagnosis of:
  - general health (screening)
  - anemia (low RBCs, small RBCs, low hemoglobin concentration)
  - bacterial infection (high WBCs)
  - drug toxicity (low platelets)
  - leukemia (increased subtypes of WBCs)
  - allergy (increased subtype of WBCs)



COUNTING BLOOD CELLS



Blood Smears



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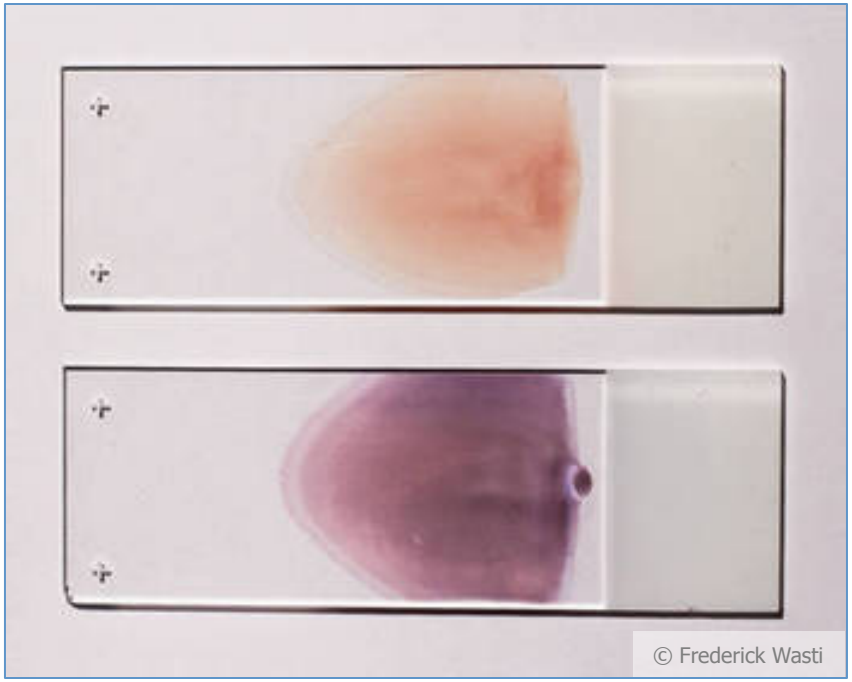

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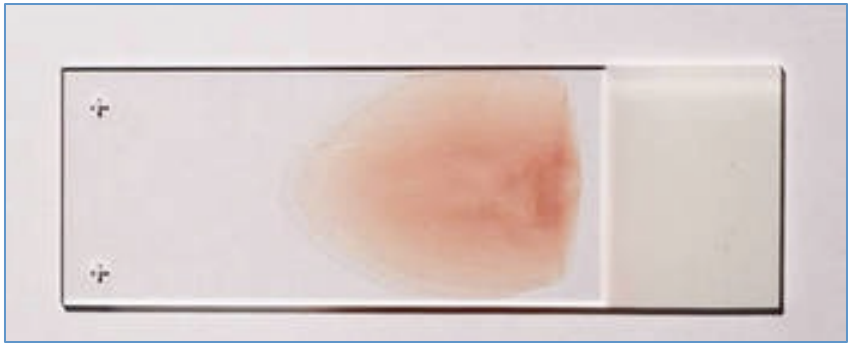
Blood Smears



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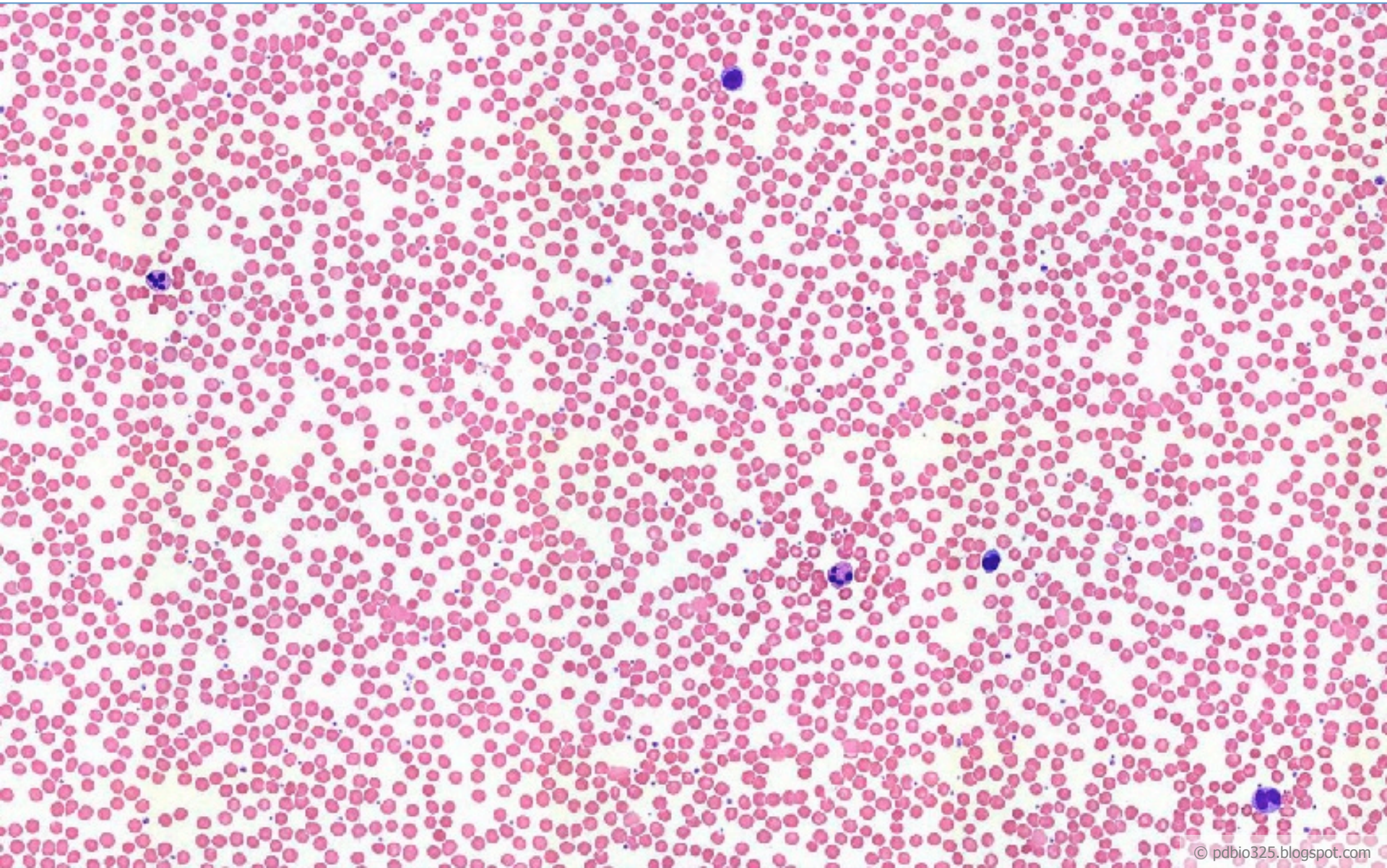
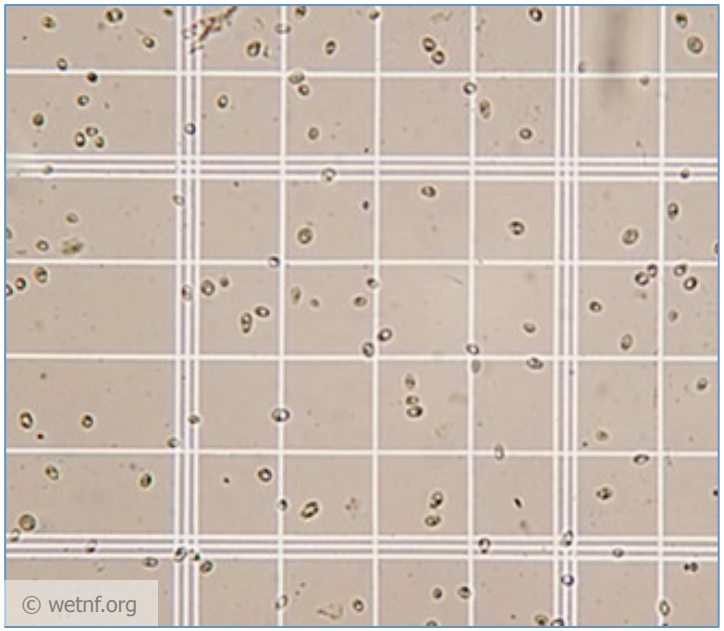
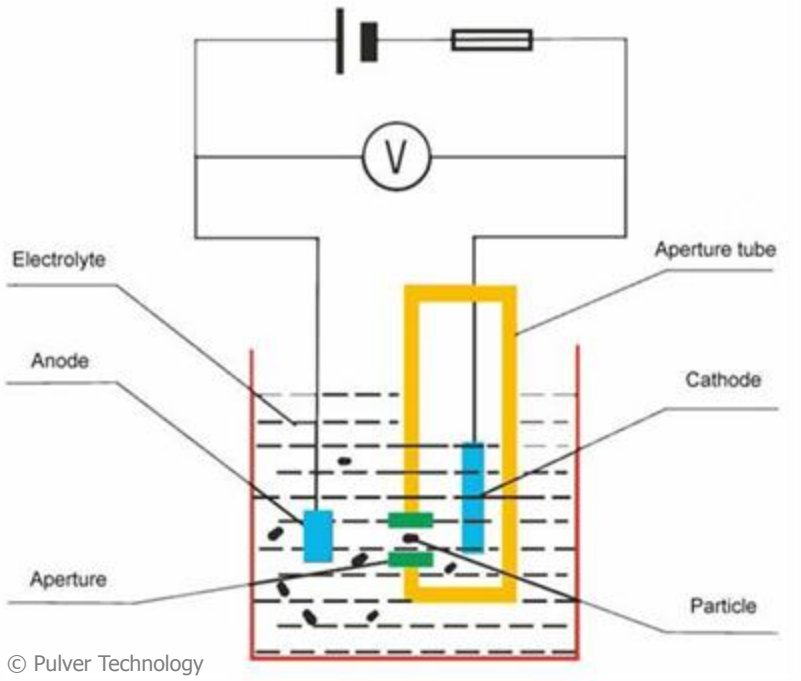
Blood Smears



Hemocytometer: Manual Counting



Particle Sizing: The Coulter Principle



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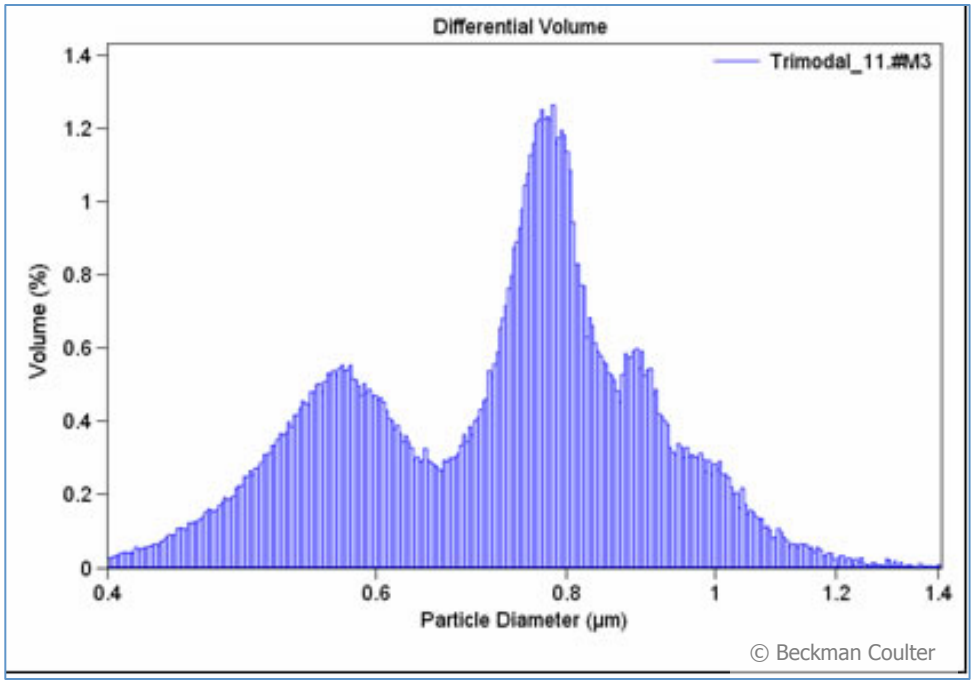

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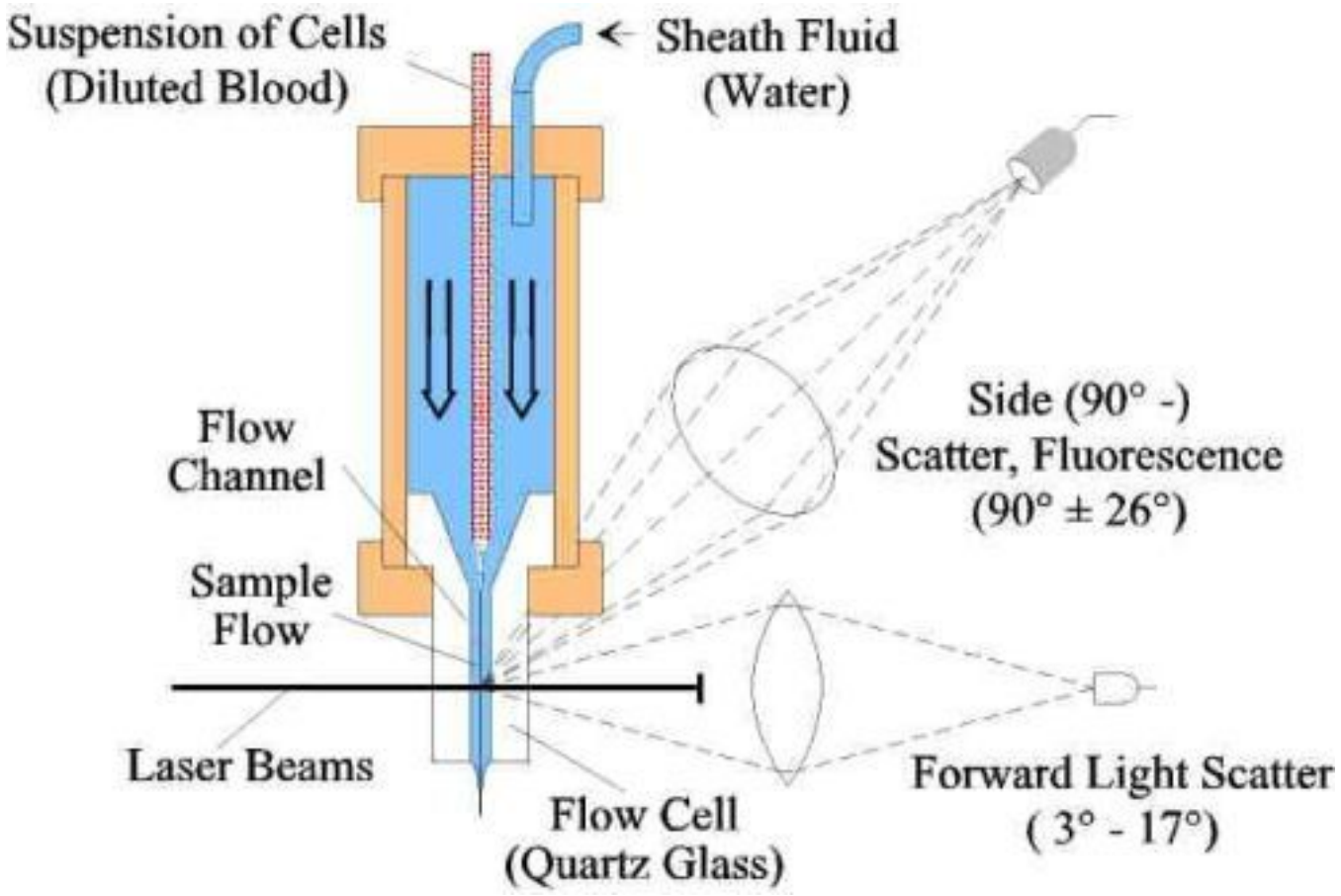
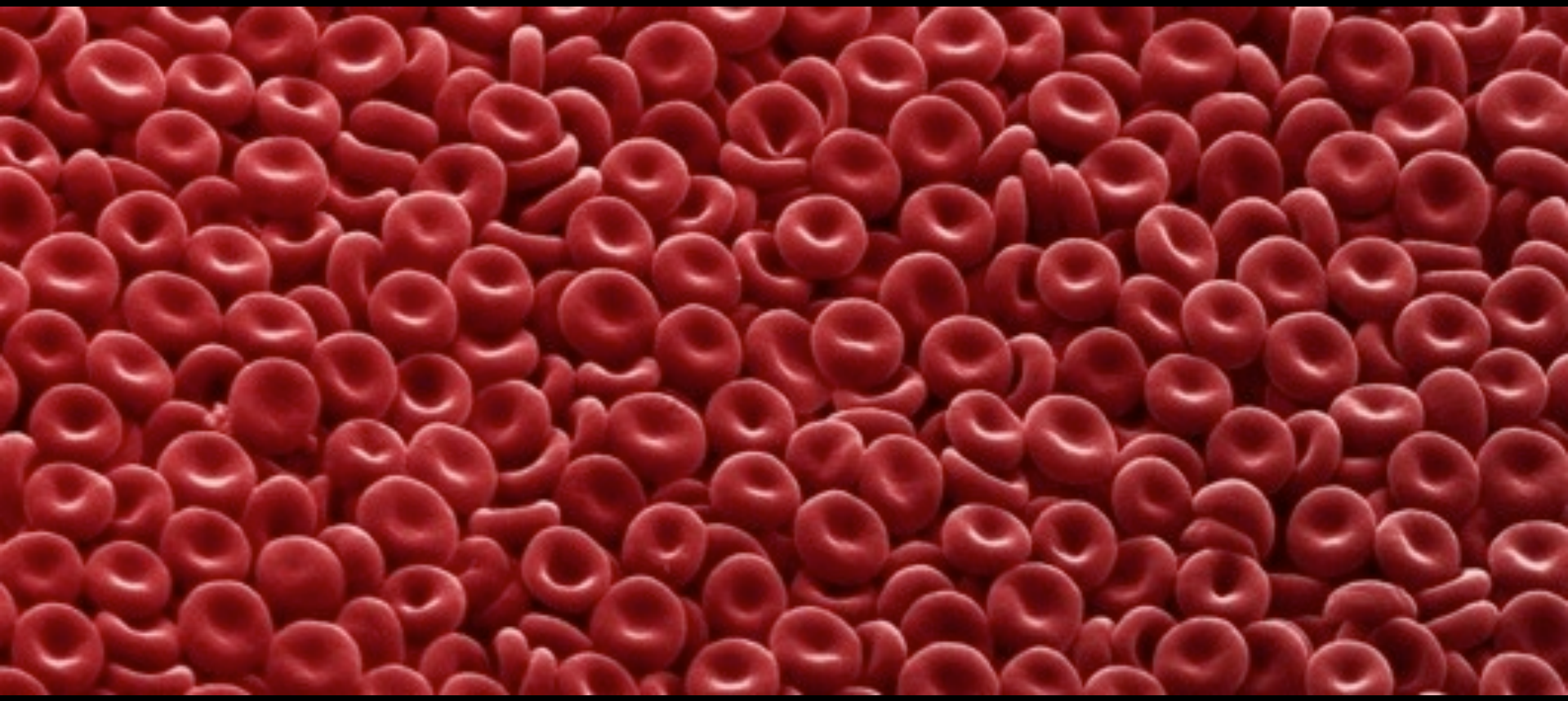
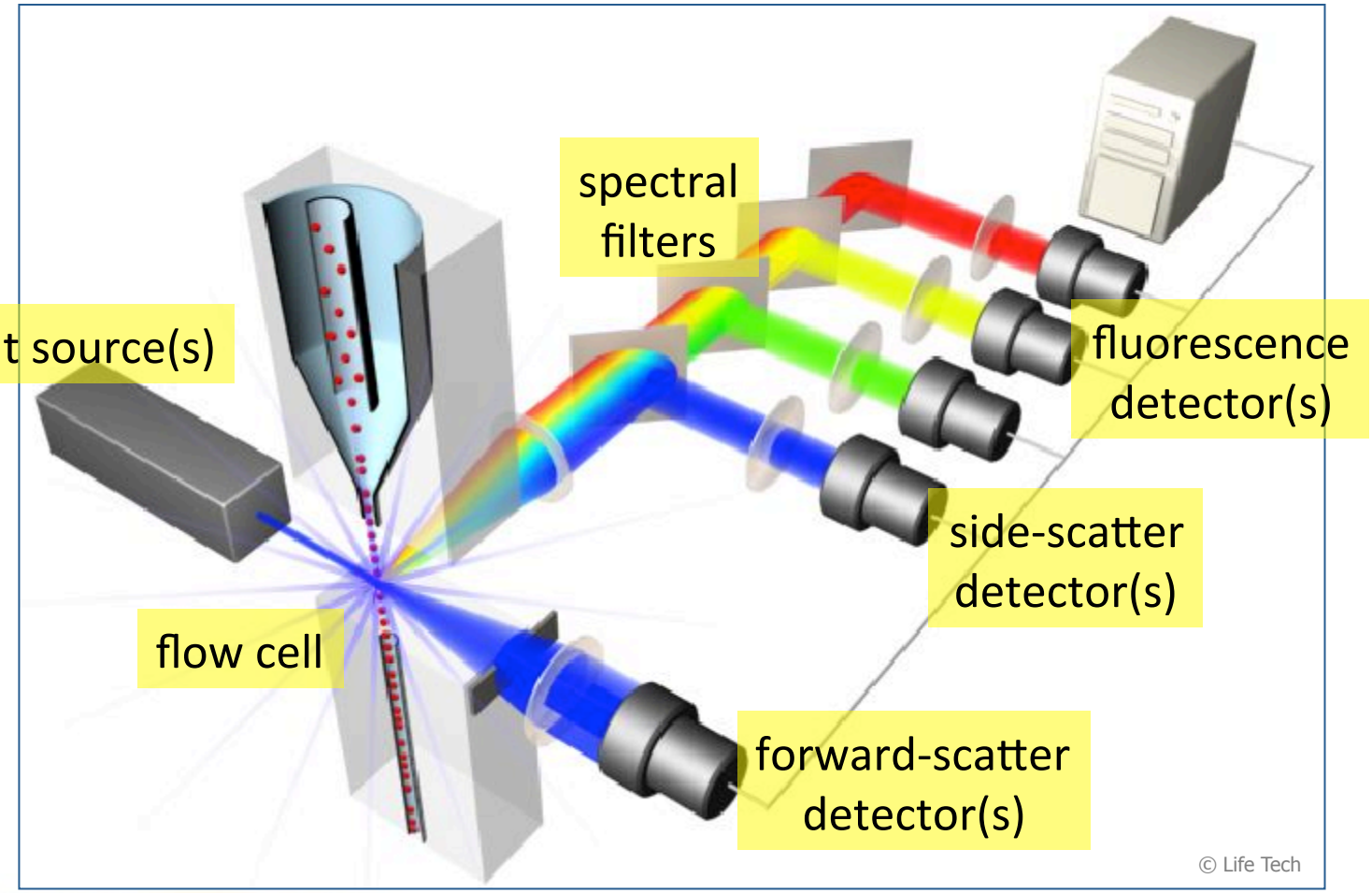
Cell Identification: Flow Cytometry



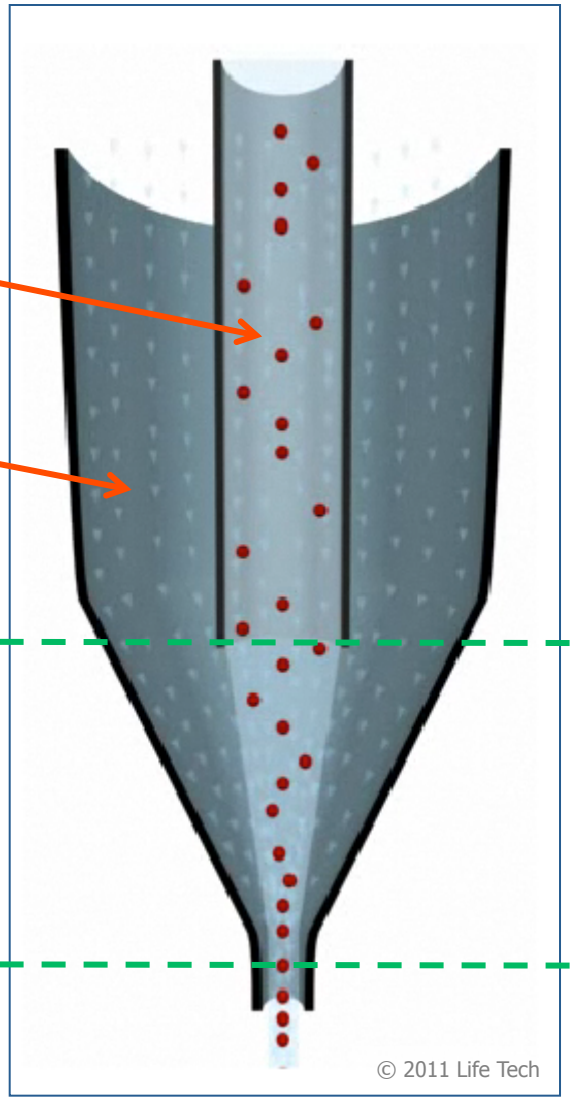
PRINCIPLES OF FLOW CYTOMETRY



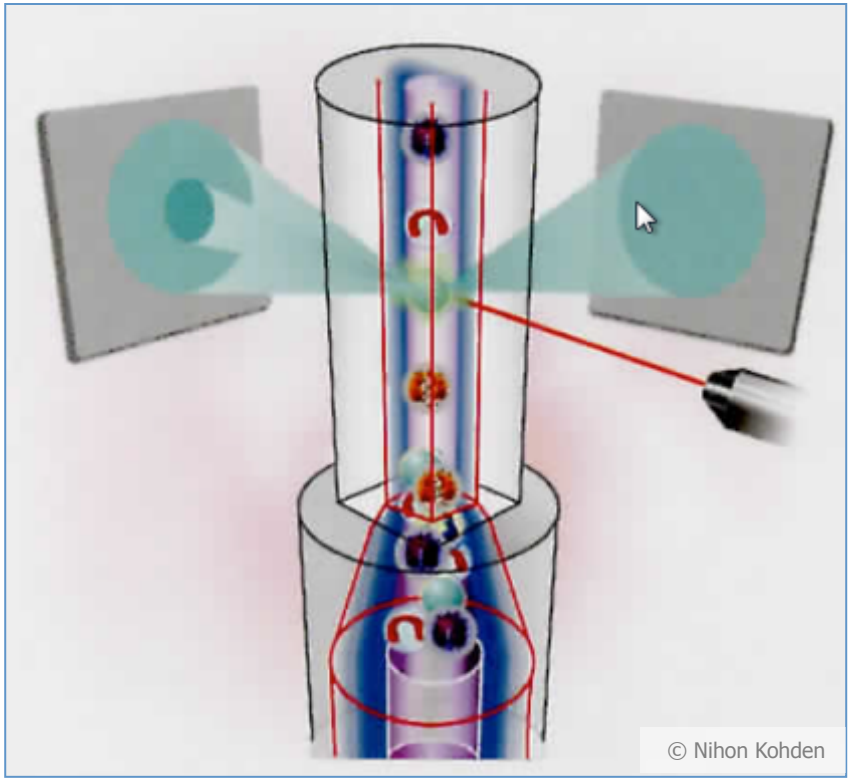
The Optical "Bench:" Critical Elements



Hydrodynamic Focusing

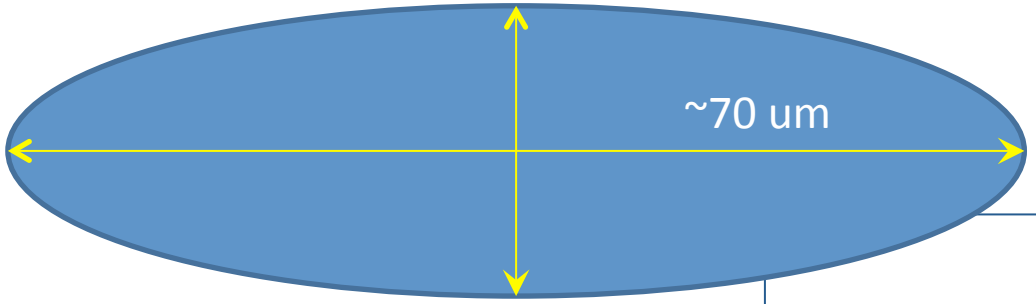


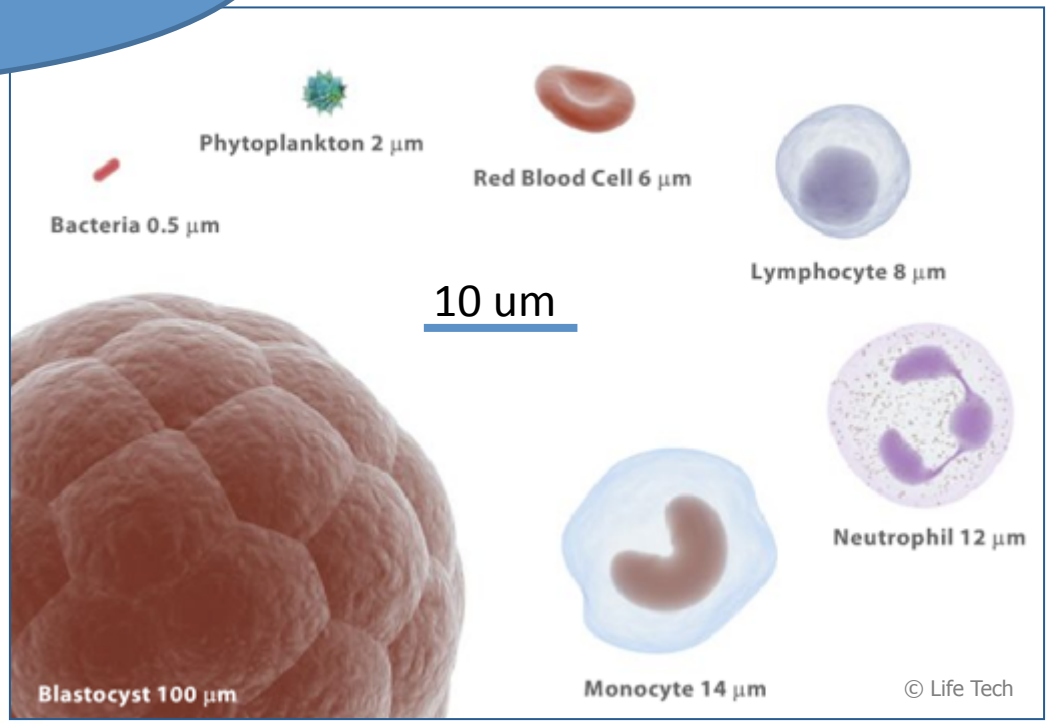
Flowcell: Where It All Comes Together



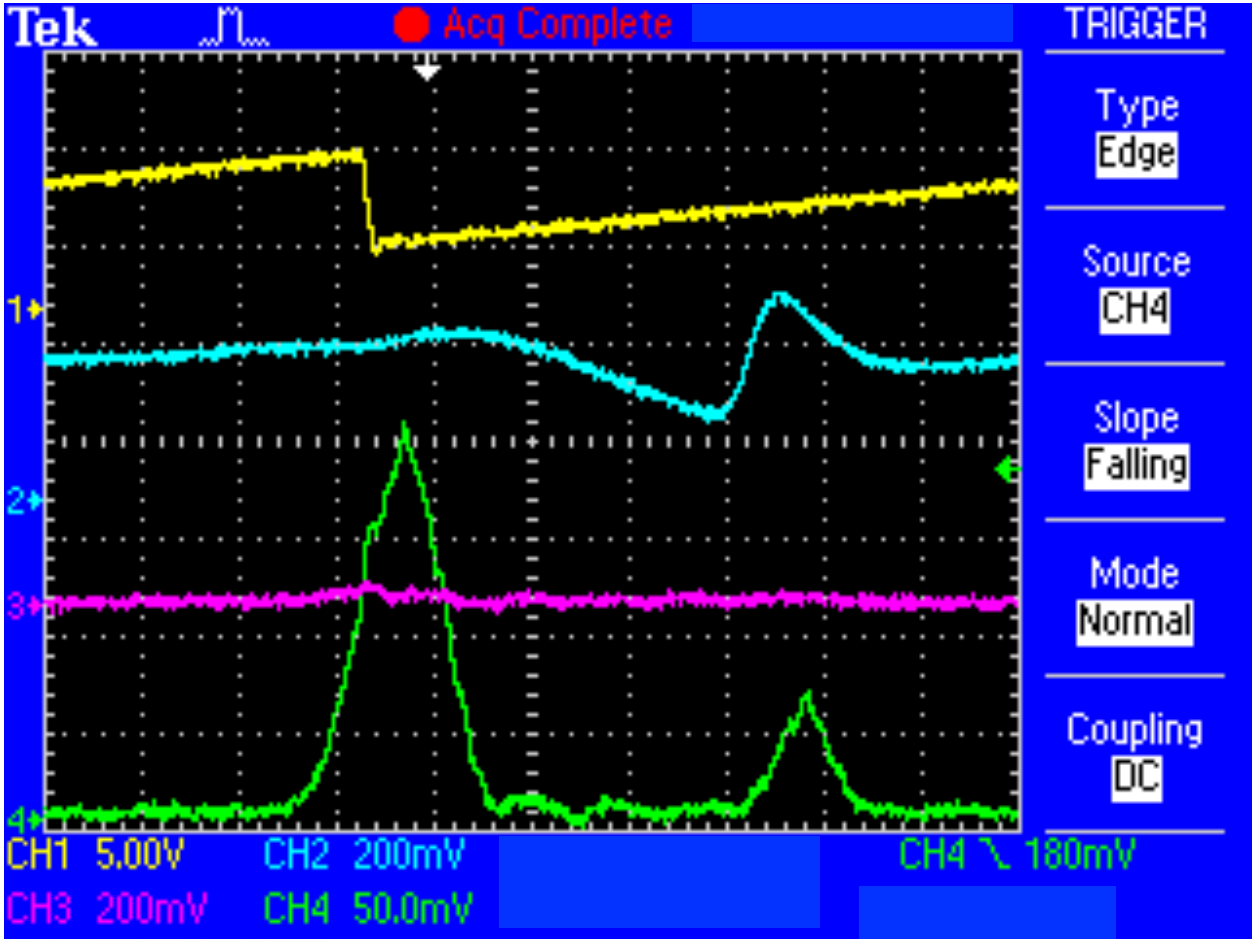
Cell Interrogation: Relative Sizes

$1/e^2$ laser beam waist

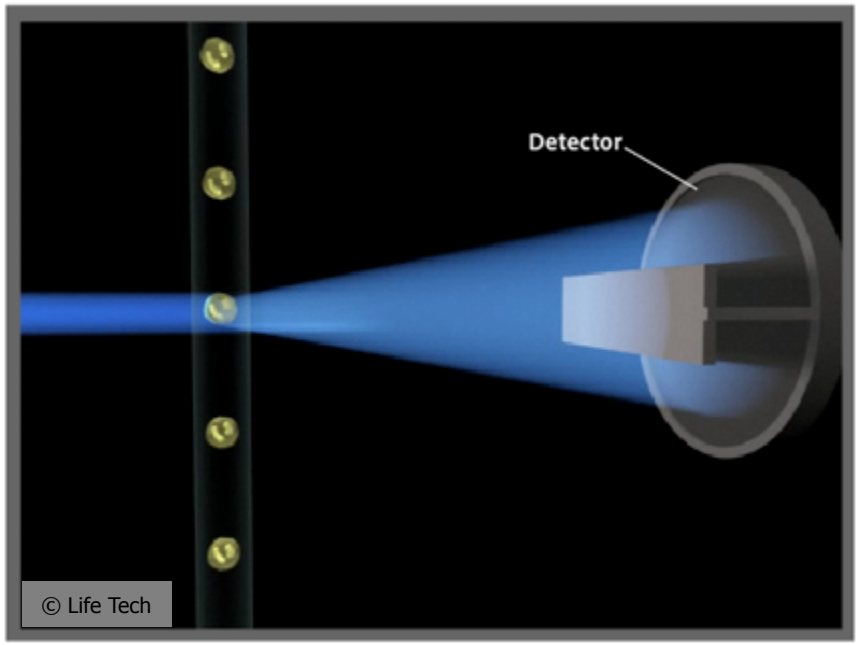


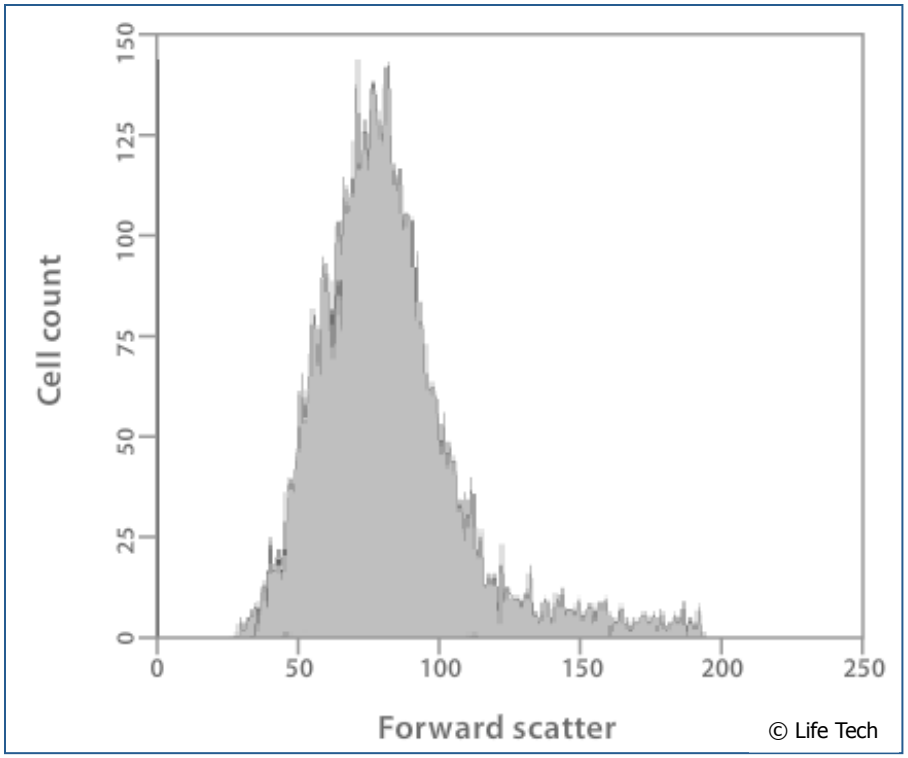


Particle Detection: Scattering Signal

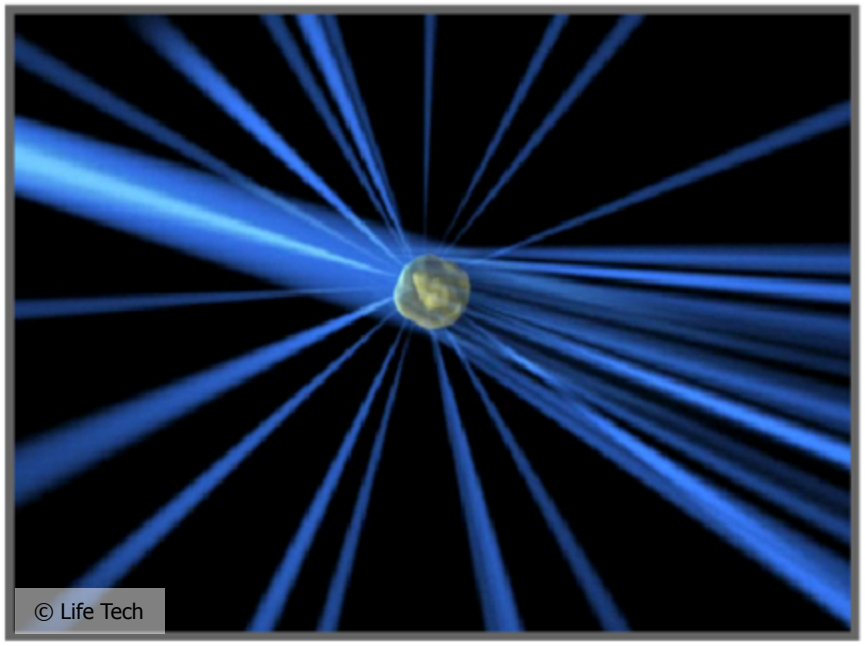


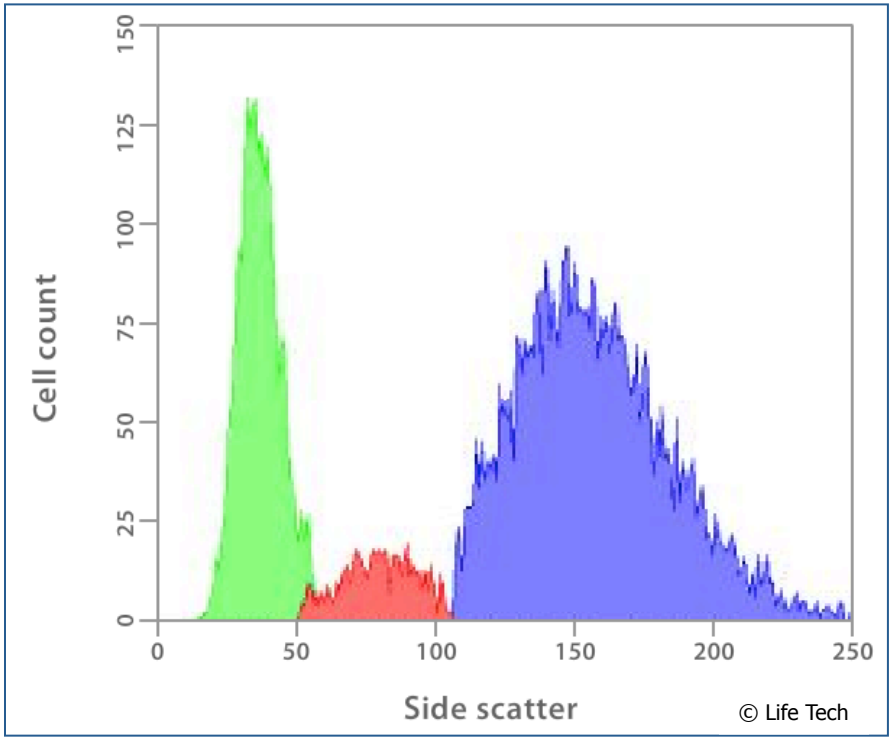
Forward Scattering Histogram





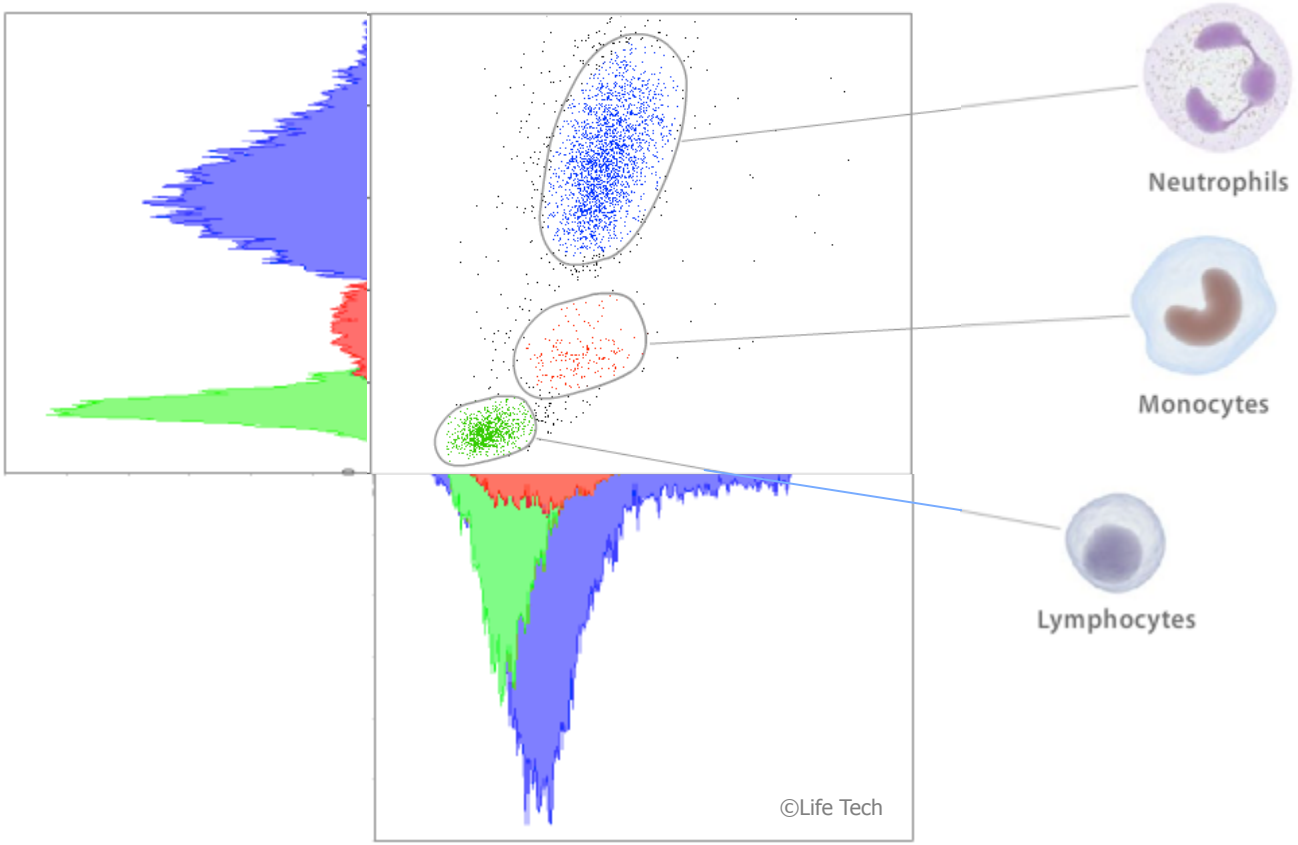
Side Scattering Histogram





Building the 2-D Scattergram

side scatter



forward scatter

Two Methods: Scattering & Fluorescence

- Scattering: mainly hematology analysis FC
  - cell size
  - morphology (shape of cell and nucleus)
  - complexity (presence of crystals, granules)
- Fluorescence: mainly non-hematology FC
  - DNA, RNA staining
  - surface antigens (immune system)
    - e.g.: CD4+ lymphocytes for HIV monitoring
  - intracellular biomarkers

Two Methods: Scattering & Fluorescence

- Scattering: mainly hematology analysis FC **$3B/yr**
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This Seminar: Scattering, Hematology

- Scattering: mainly hematology analysis FC **$3B/yr**
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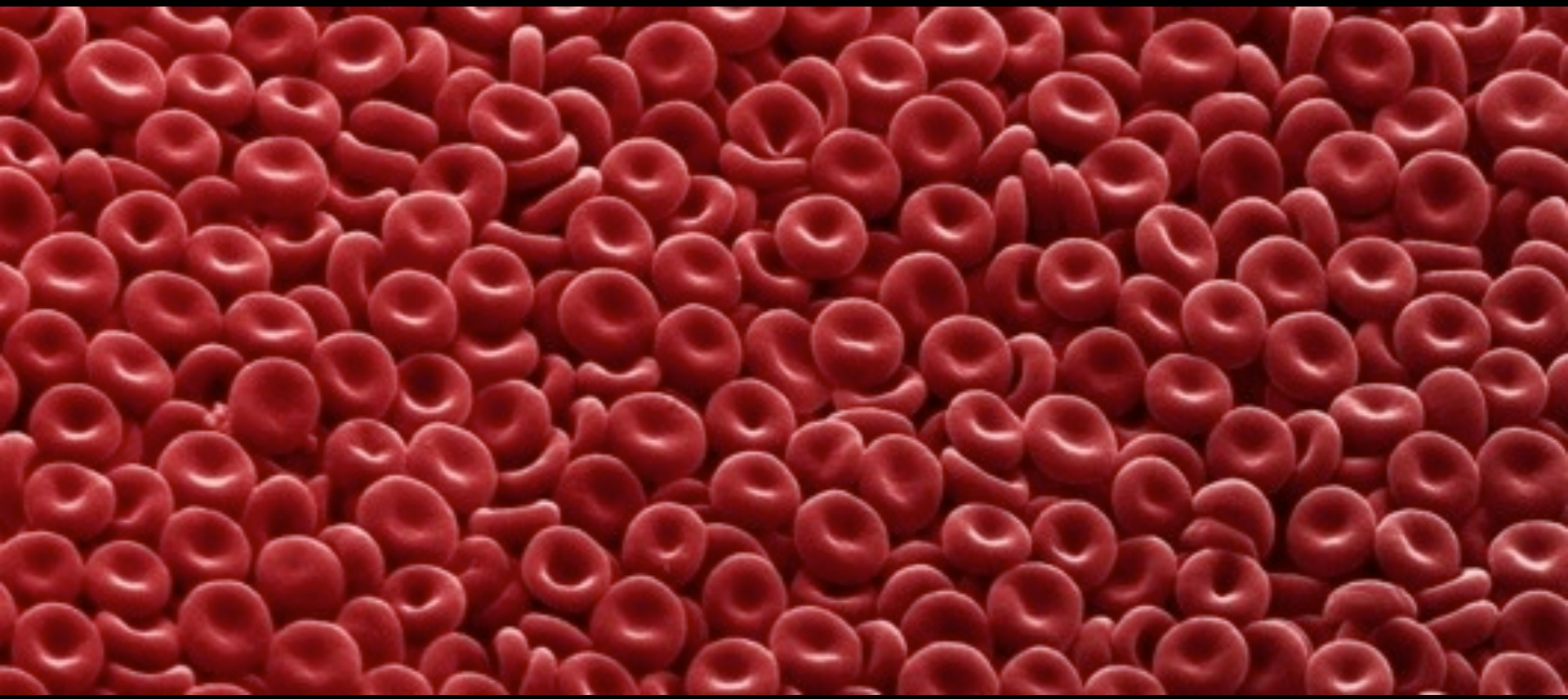
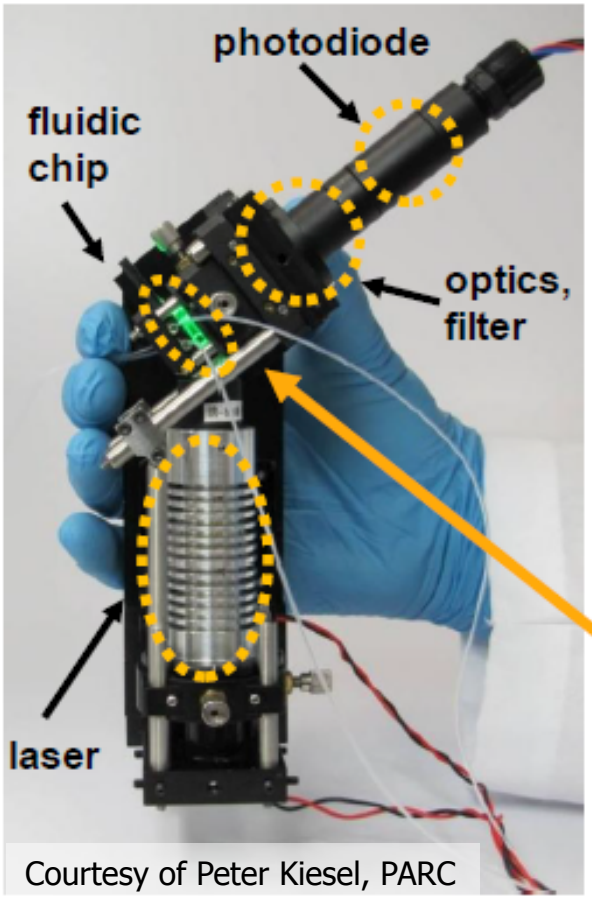
FLOW CYTOMETRY SYSTEMS



From Very Small…



Courtesy of Peter Kiesel, PARC

…To Very Large

Hematology Analyzer Examples


© Beckman Coulter


© ABX


© Orphee


© Abbott Laboratories

Flow Cytometer Examples

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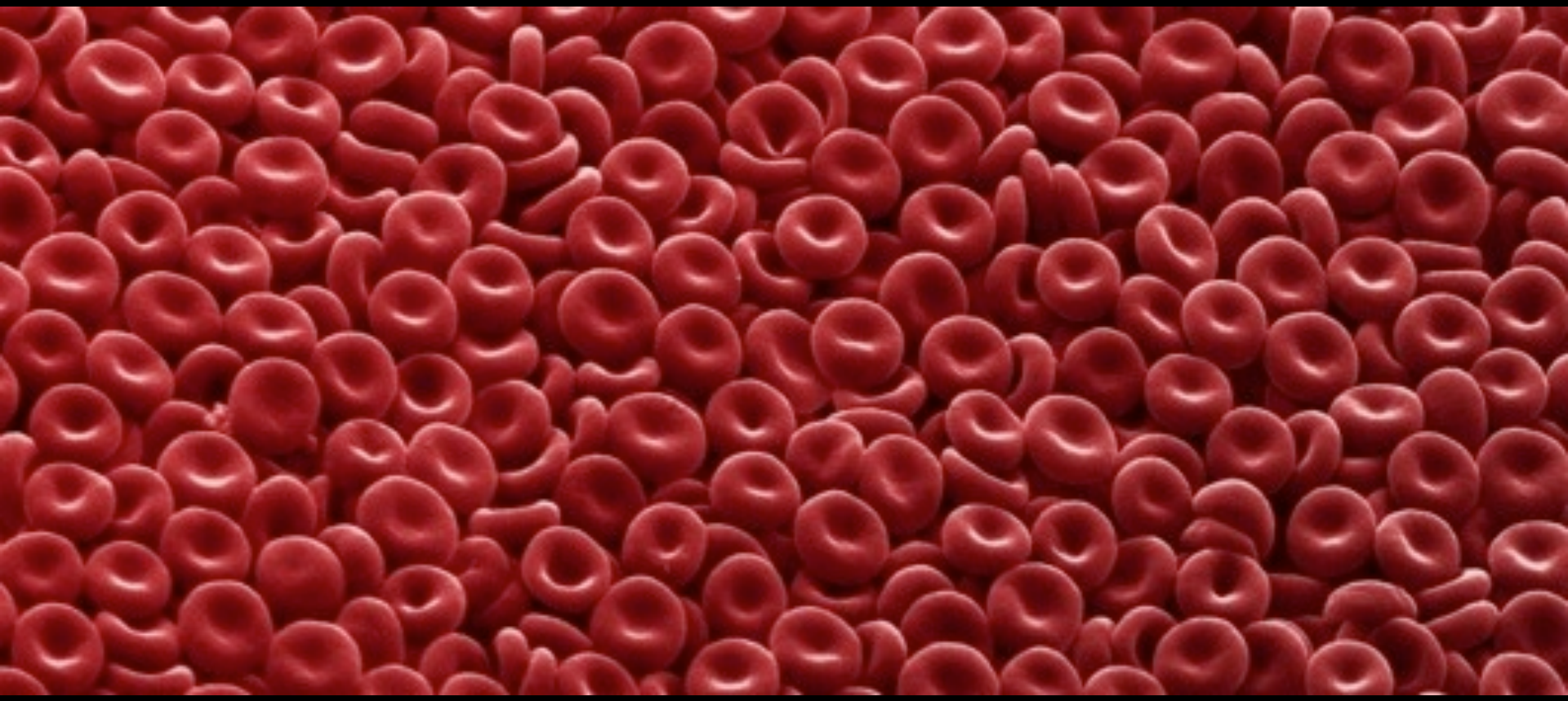
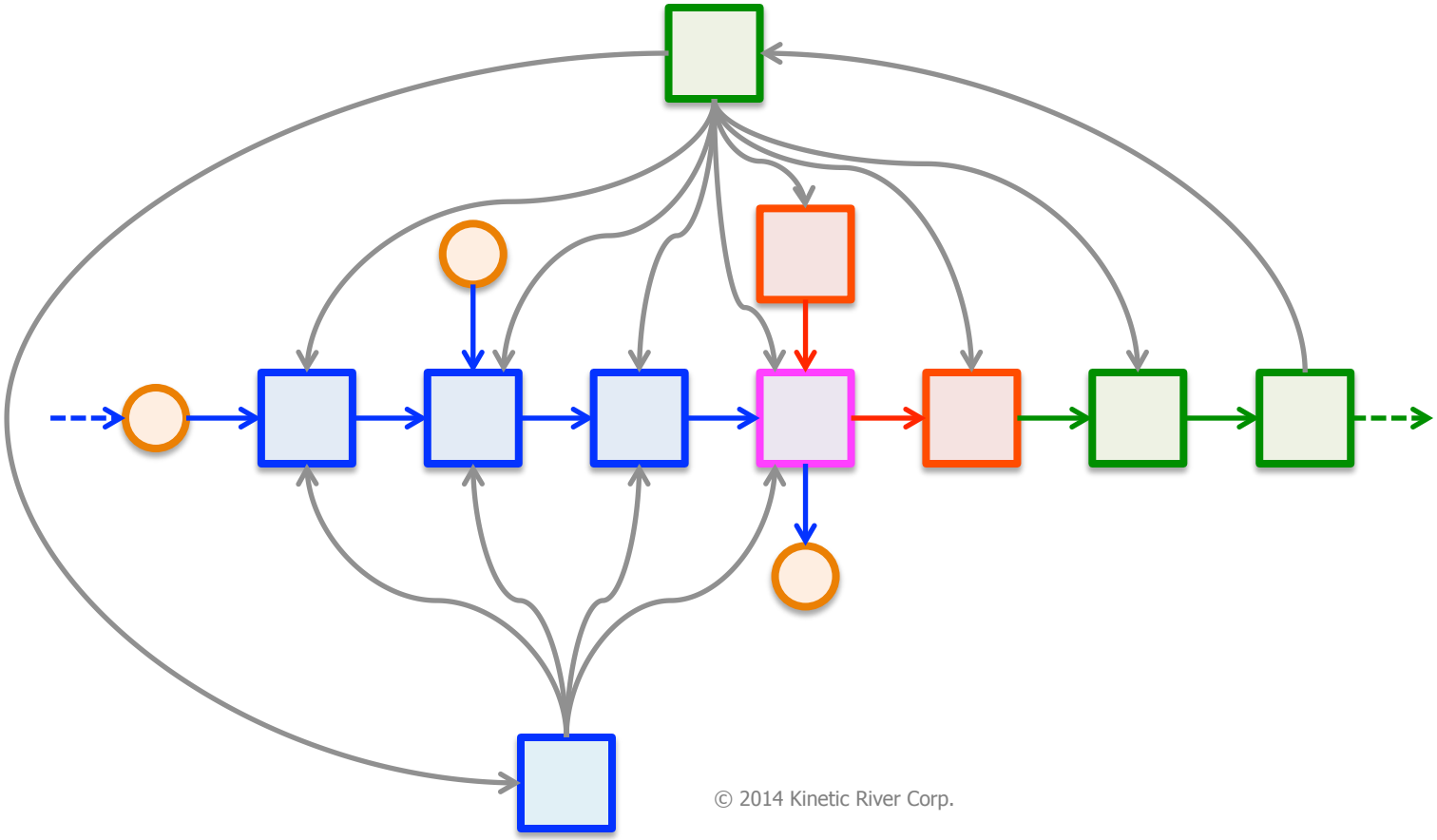
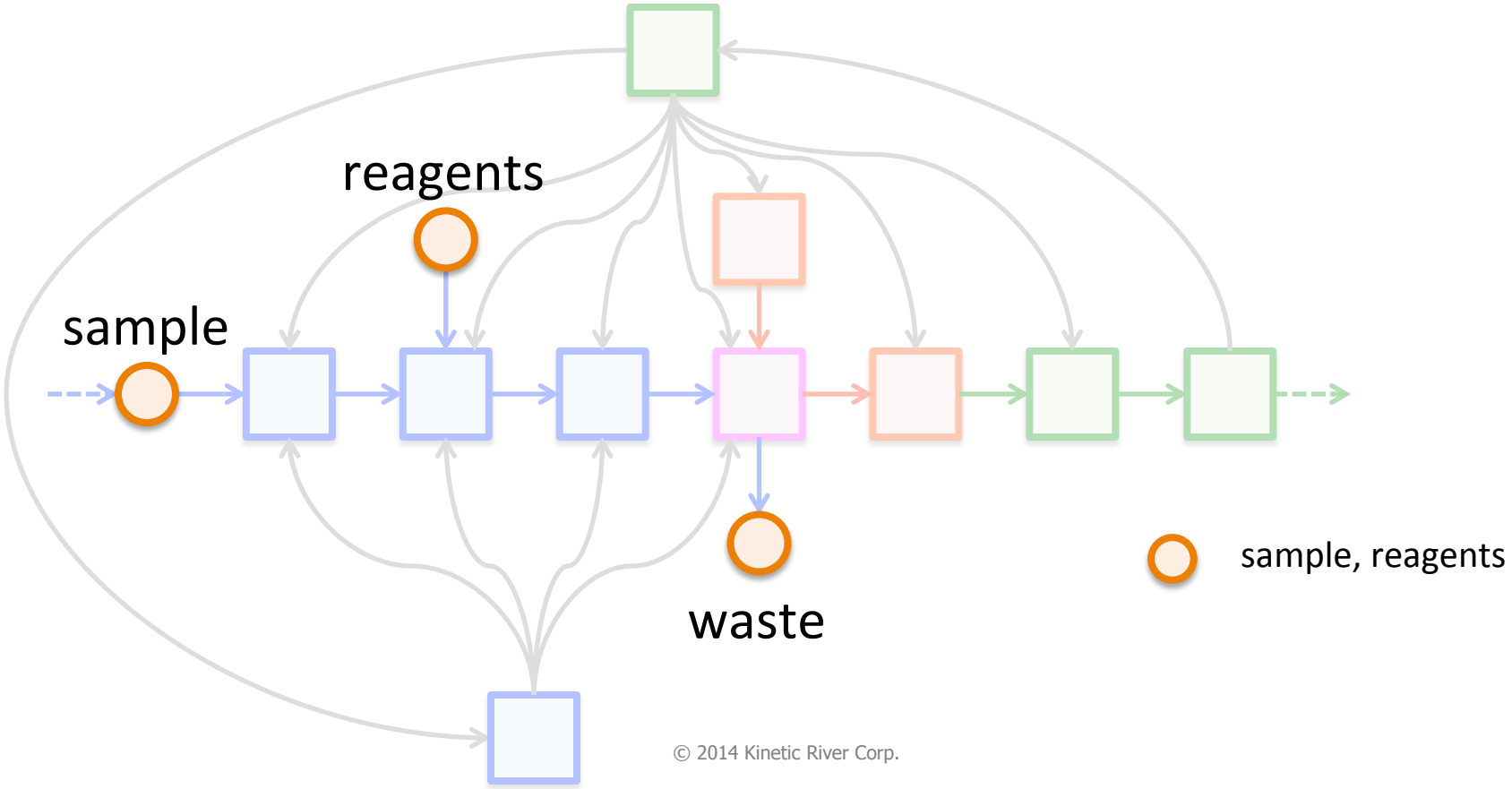
FLOW CYTOMETRY SYSTEM ARCHITECTURE



Flow Cytometer HW Modules



Flow Cytometer HW Modules



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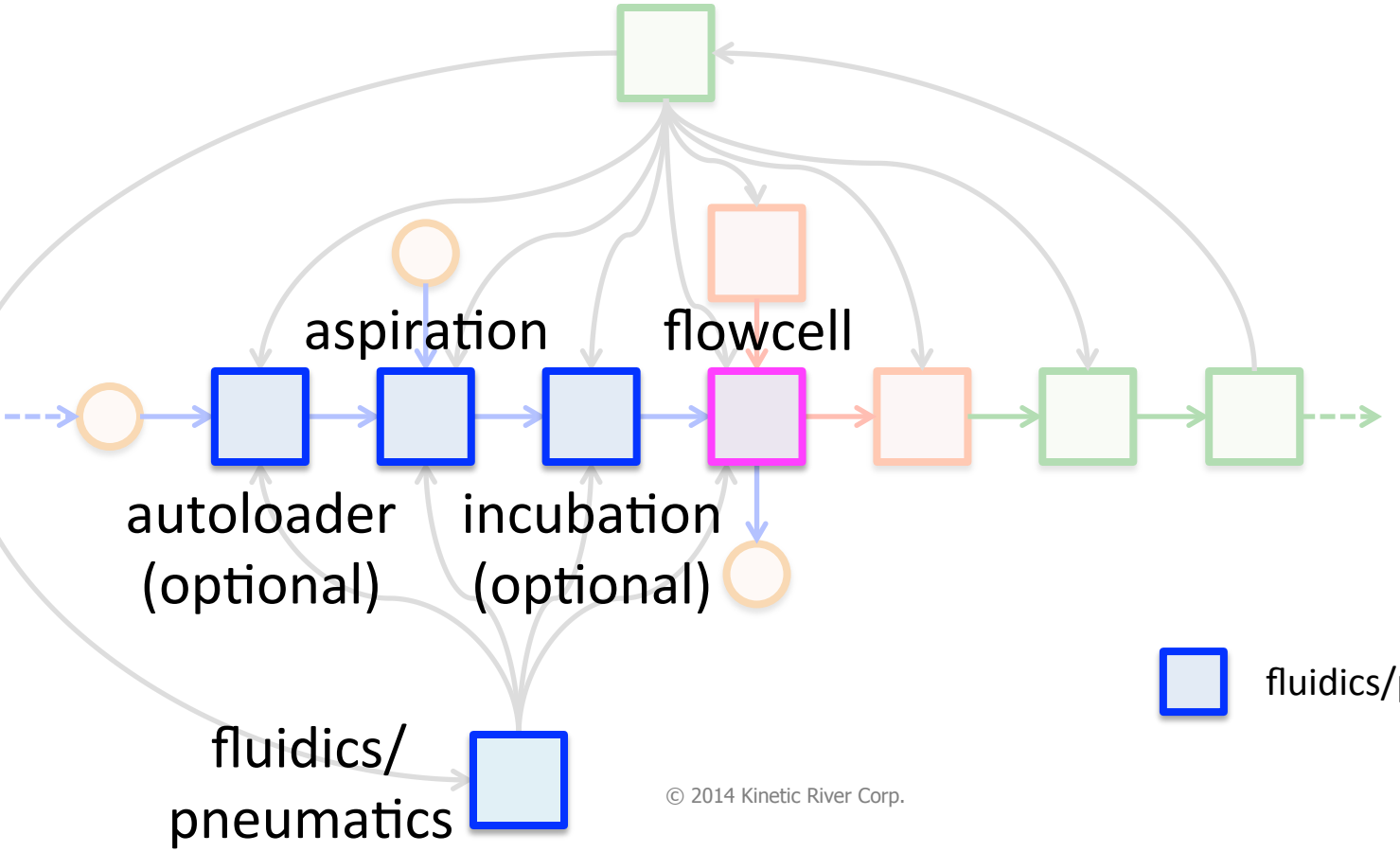

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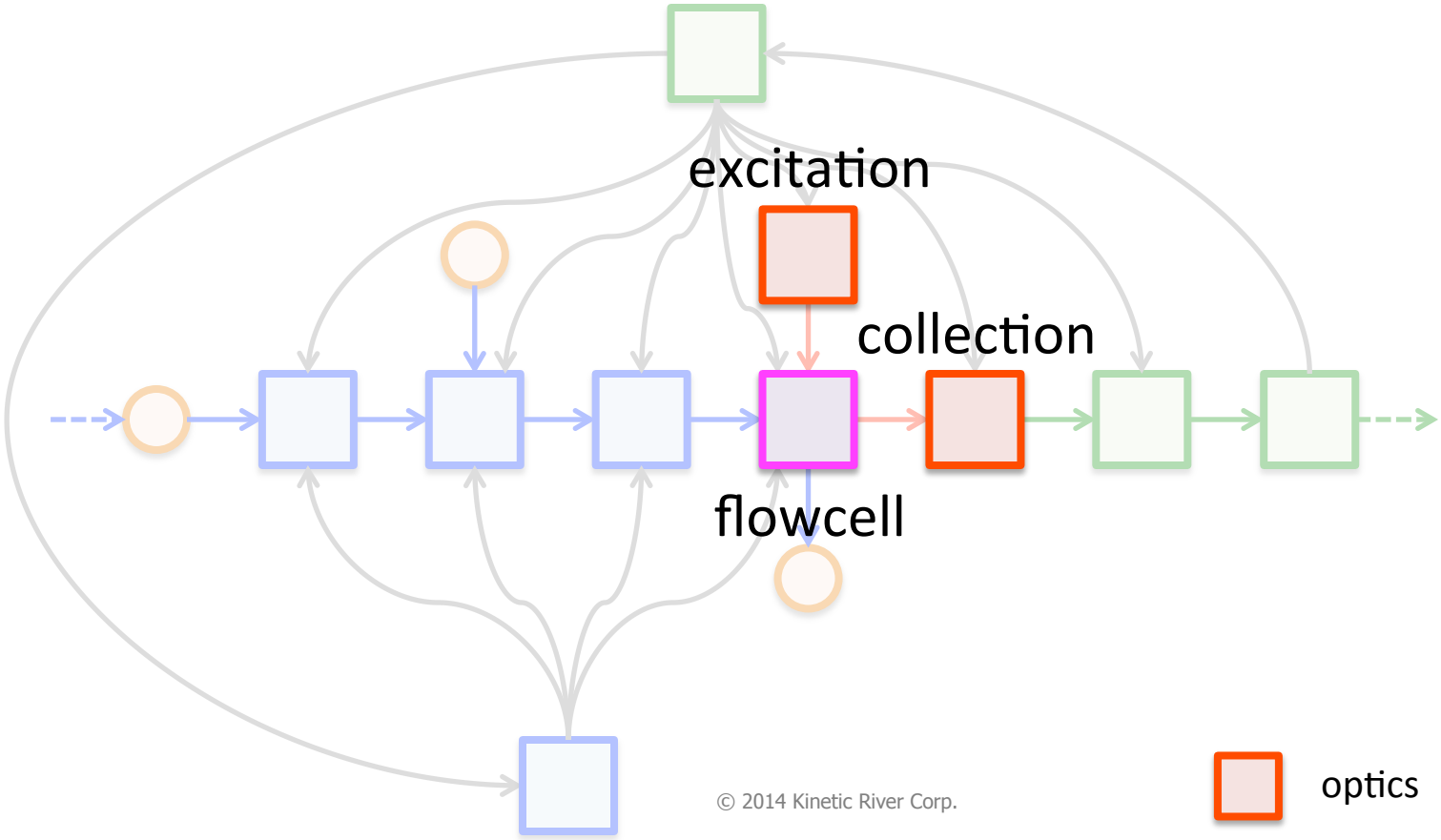

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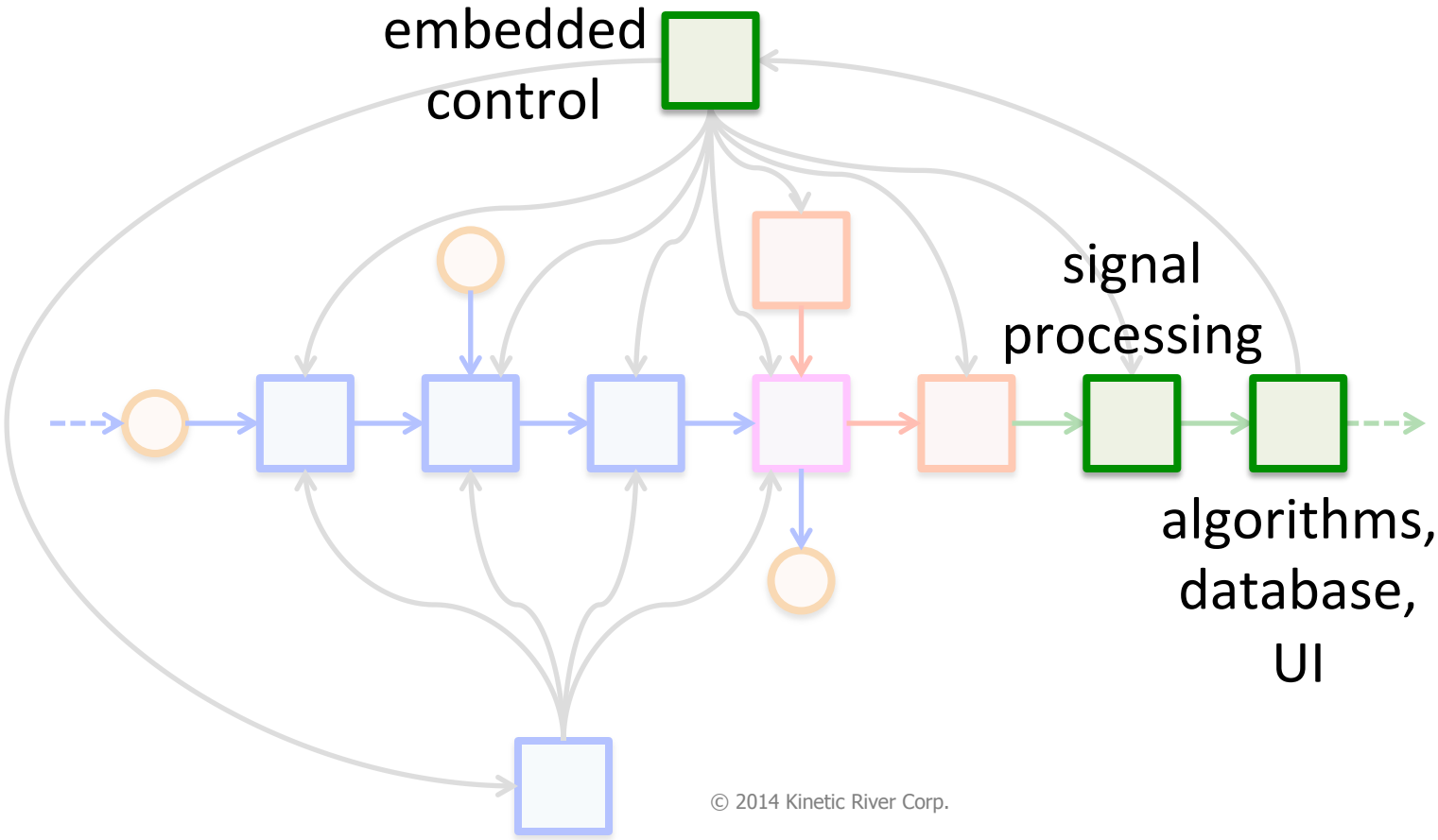
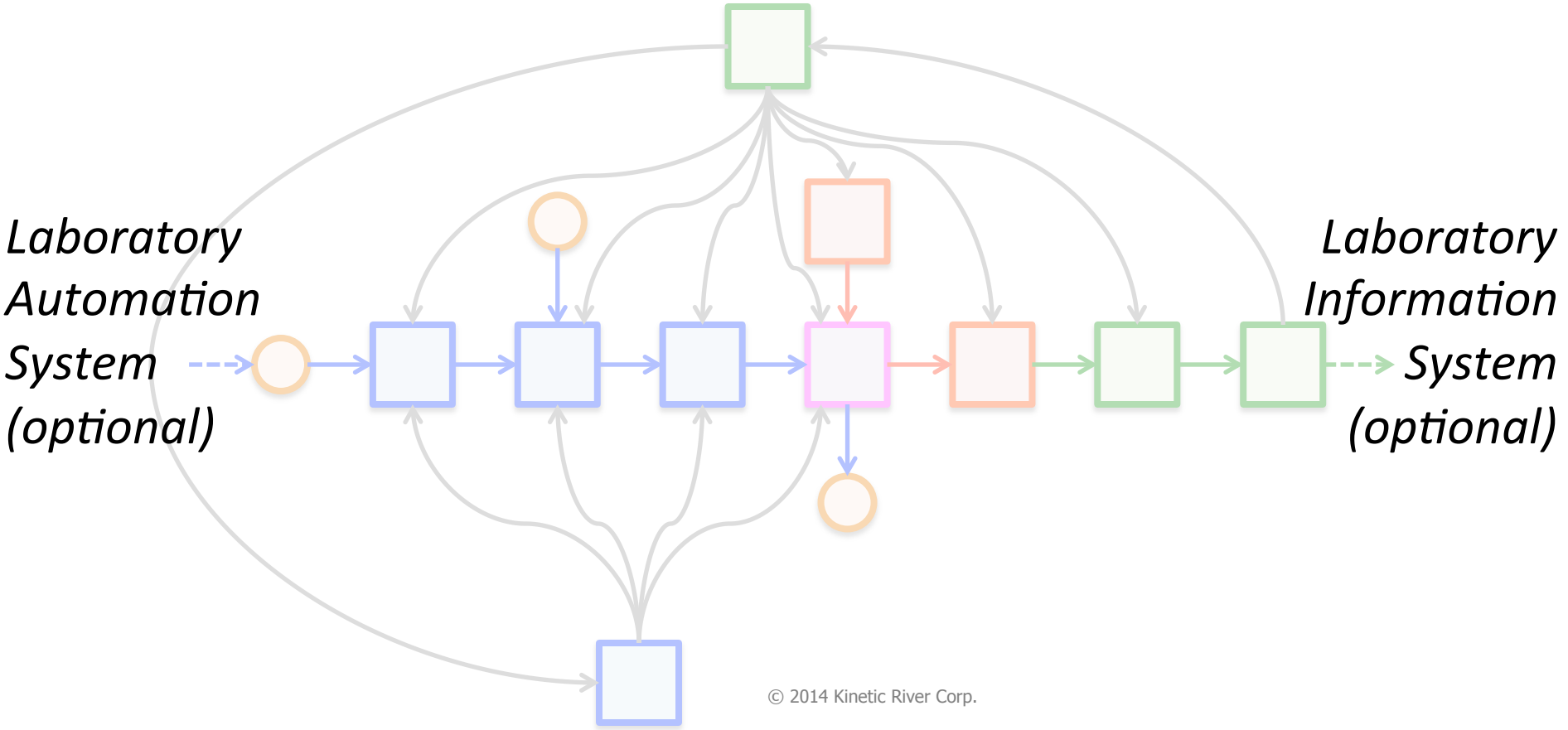
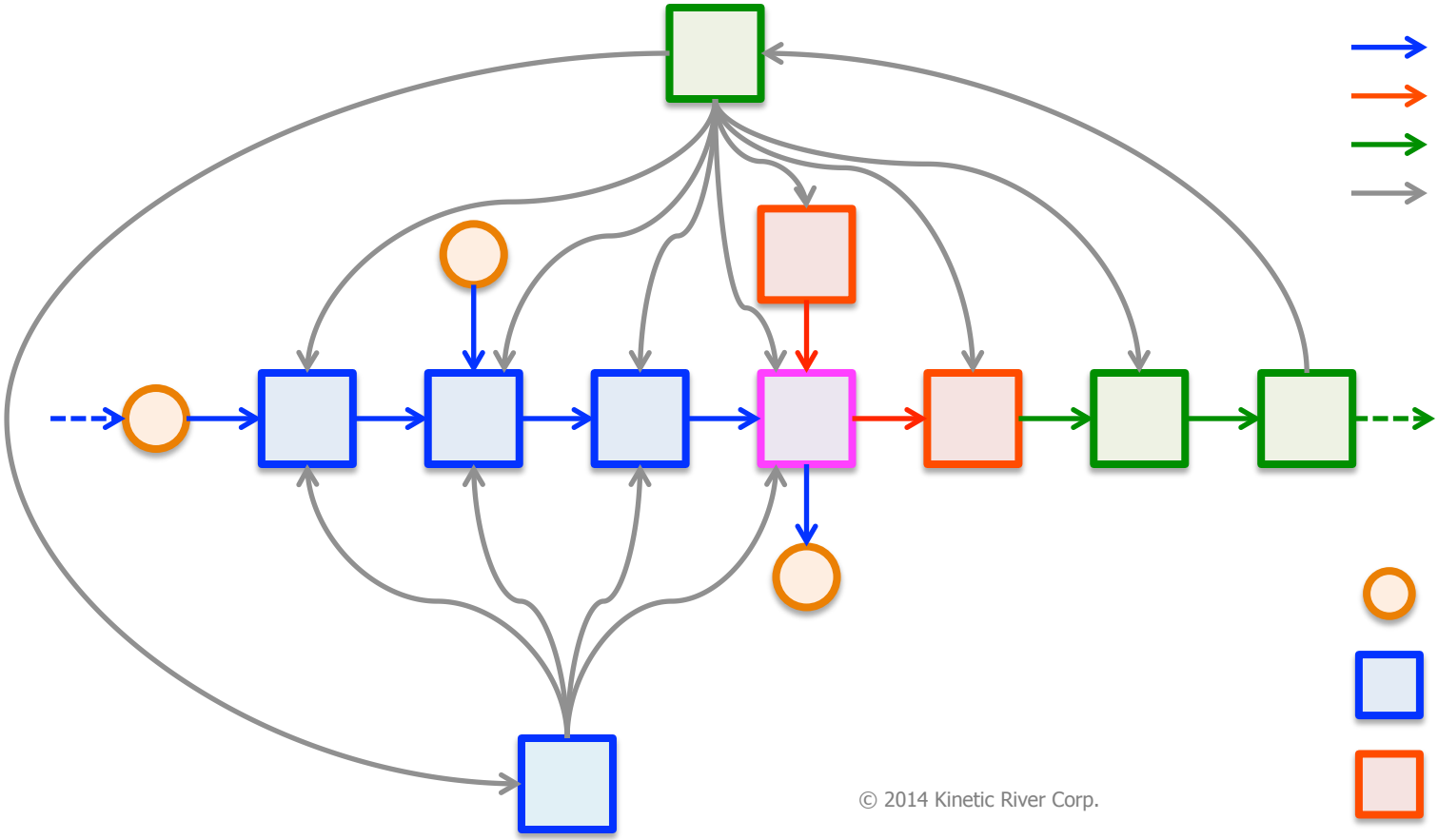


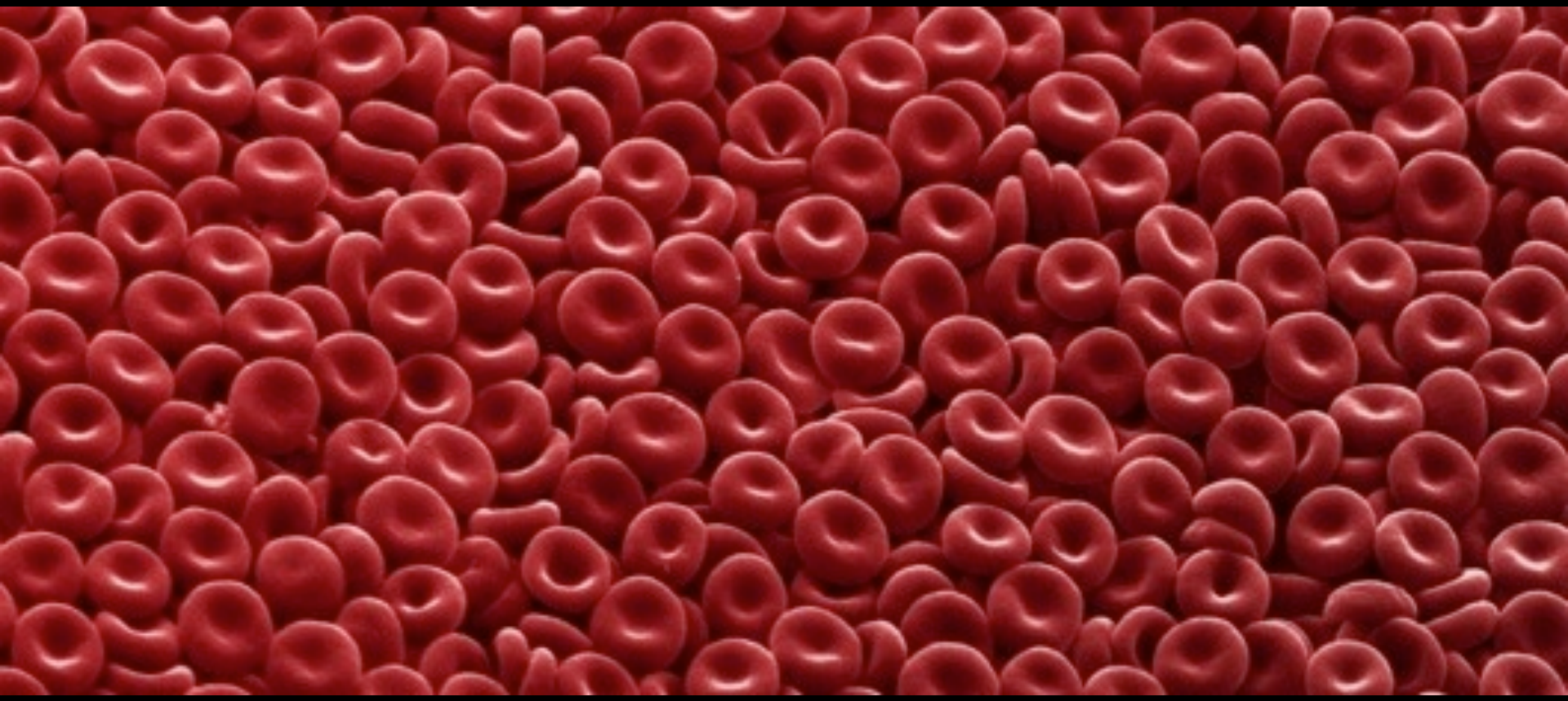
Flow Cytometer HW Modules



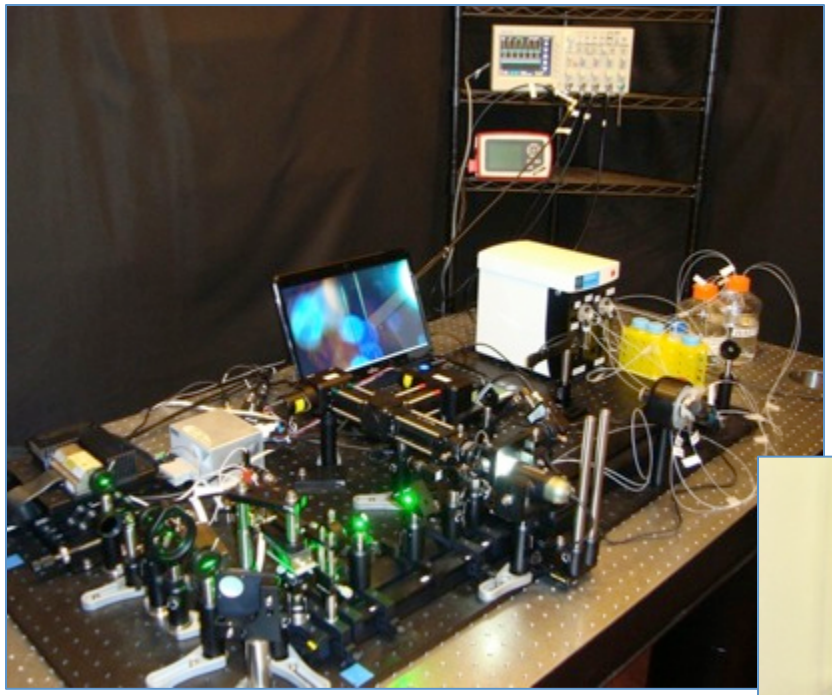
Flow Cytometer HW Modules



DEVELOPING FLOW CYTOMETERS

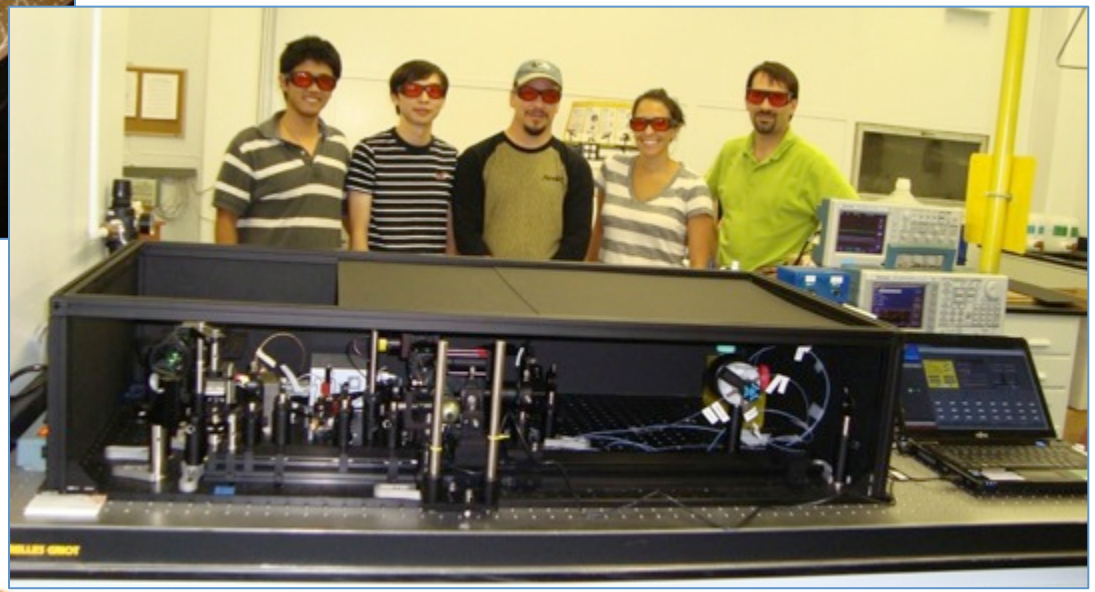


Flow Cytometers Are Complex Systems



- e.g.: Kinetic River's *Danube* FC
- optics, microfluidics, control SW: **KRC**
- fluidic management
- electronics
- signal processing

PARTNERS



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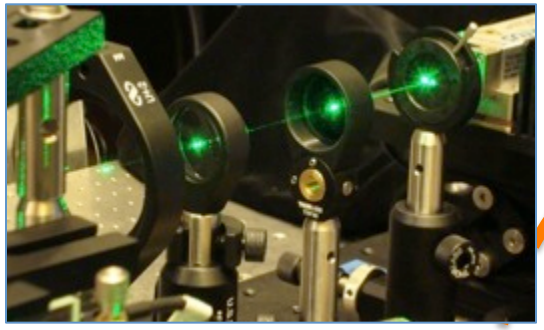
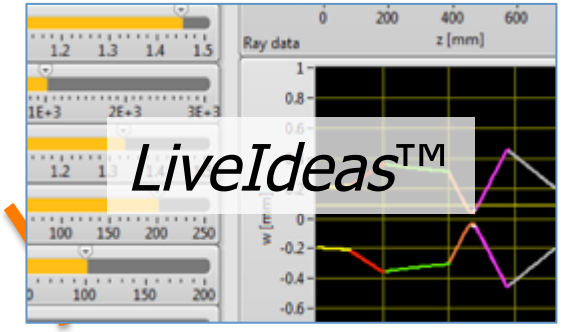
Kinetic River Key Expertise

Optics

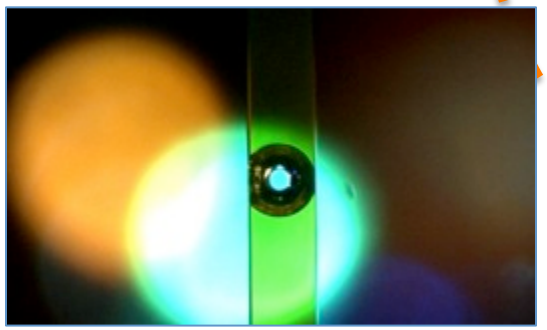


System Design

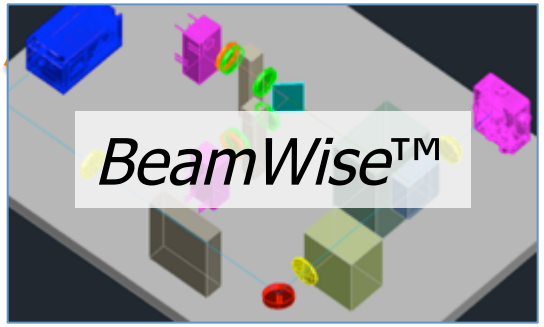


Life Sciences & Diagnostics

Microfluidics



2D / 3D CAD



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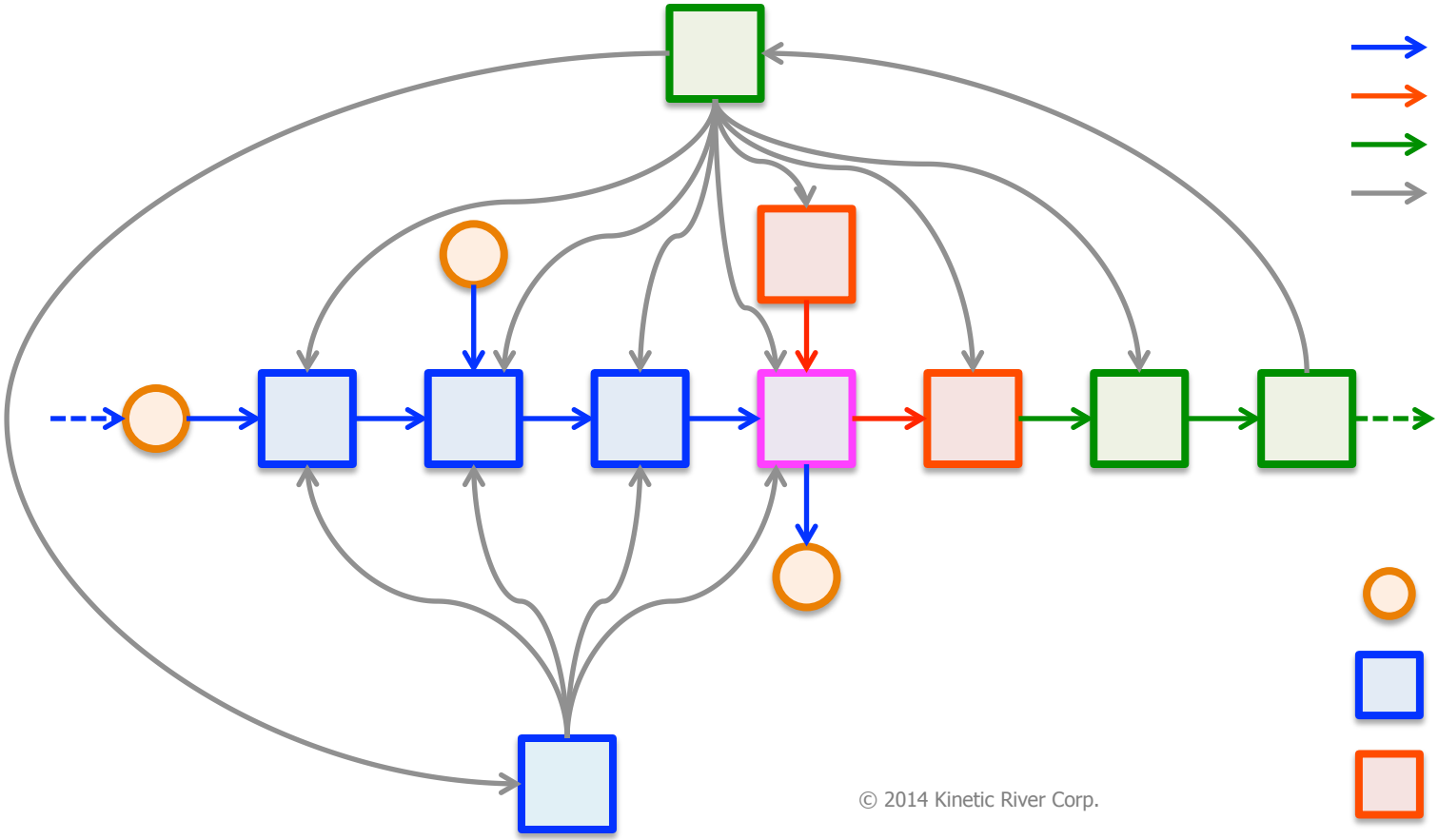
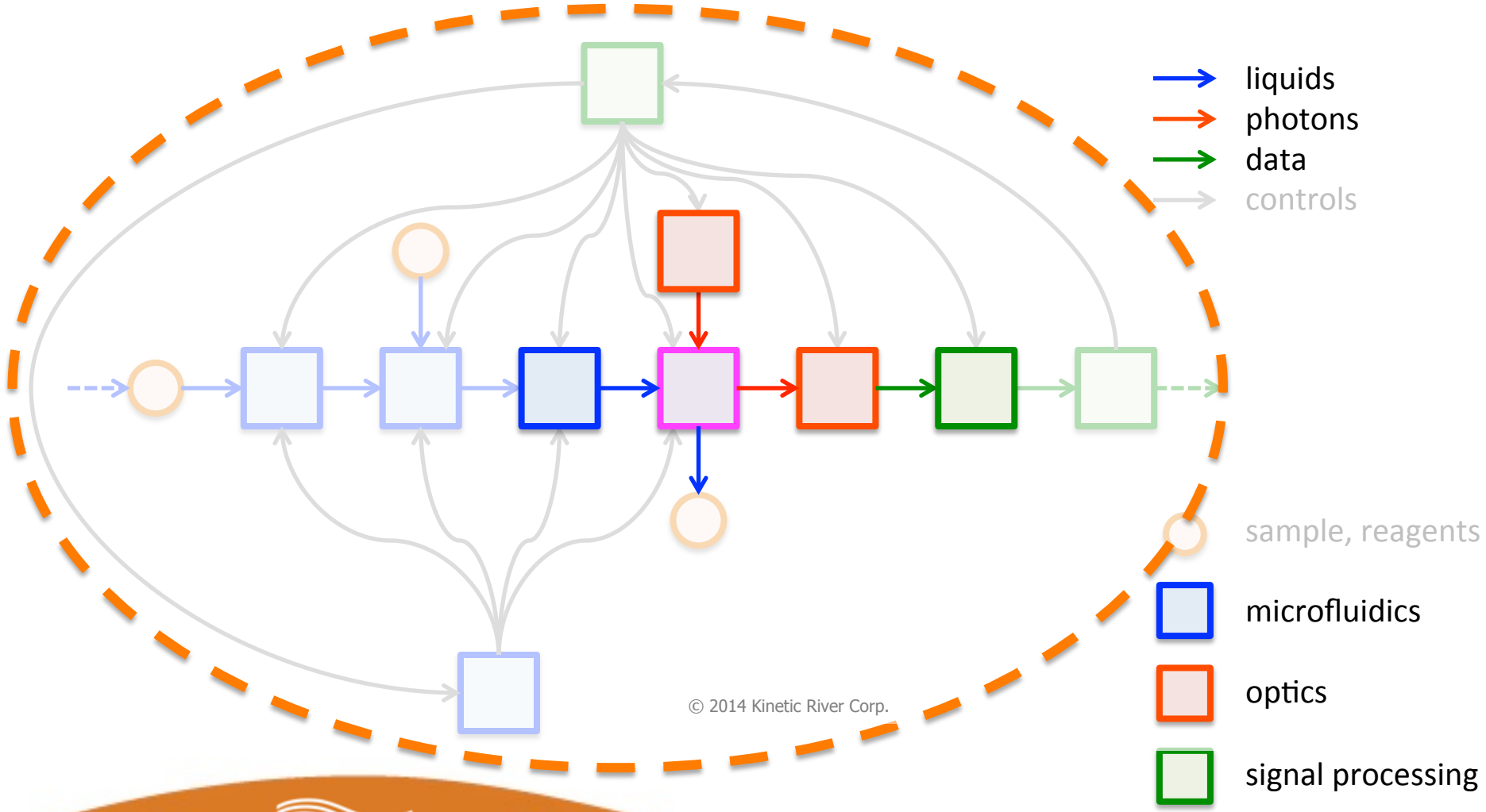
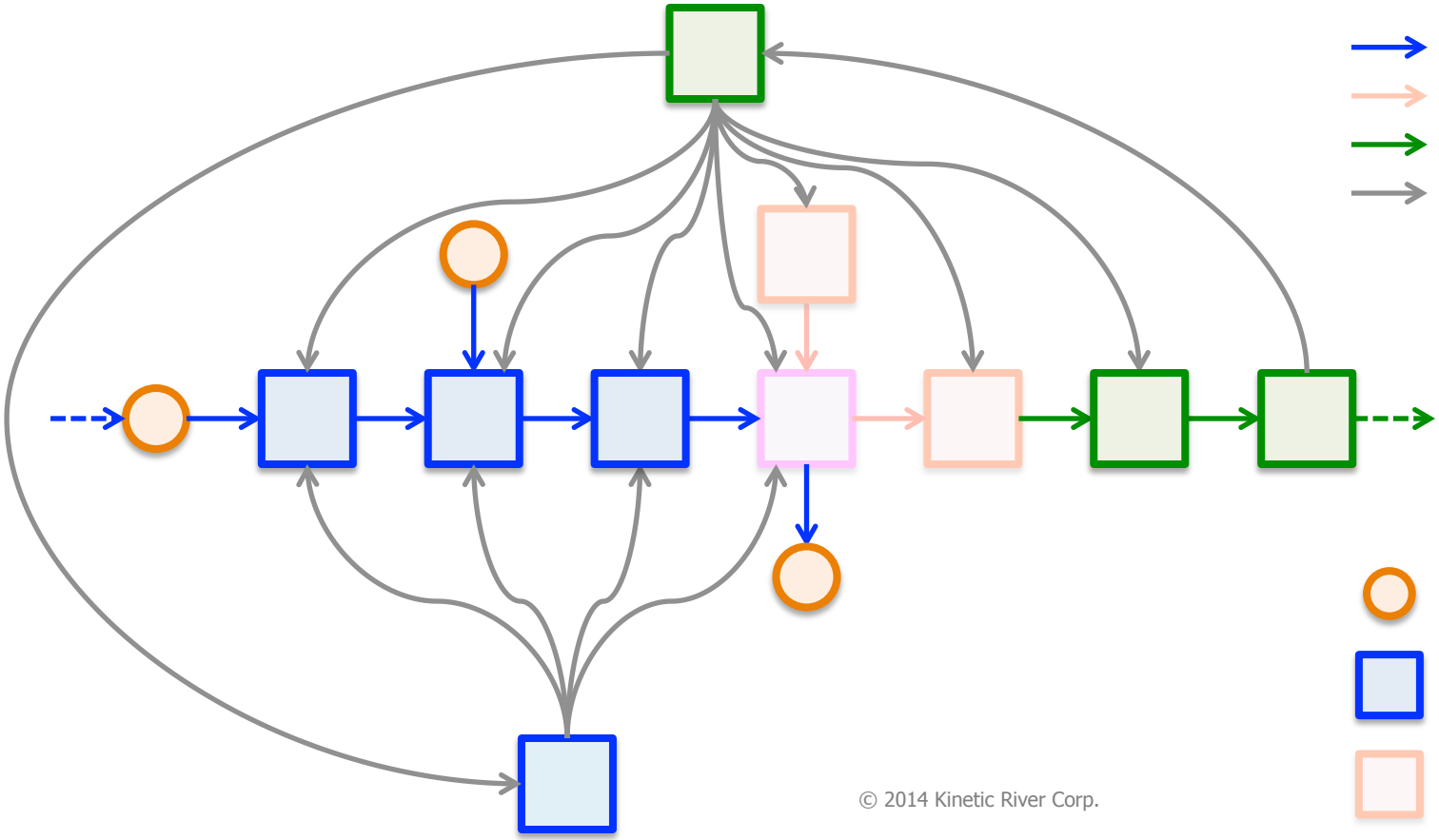
Flow Cytometer HW Modules



Kinetic River Core Competencies



Partner Core Competencies



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RESOURCES, Q&A

- Flow cytometry tutorials at www.lifetechnologies.com
- www.wikipedia.org
- Purdue University PPTs
- "*Practical Flow Cytometry*", 4th ed., H.M. Shapiro
  - also online at www.PracticalFlowCytometry.com
- "*Flow Cytometry: An Introduction*", M.G. Ormerod
  - also online at www.flowbook.denovosoftware.com
- GVacca@KineticRiver.com

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