Counting Cells using the Coulter Counter

Maggie A. Witek, Ph.D
Department of Chemistry
Center of BioModular Multiscale Systems for Precision Medicine
The University of Kansas
August 7-9, 2019
Coulter Counter Principle

...when particles pass through a narrow opening separating two electrodes they create an increase in resistance in the electrical circuit that is proportional to particle volume...

Resistance plotted against time:
- counting spikes will indicate number of particles traversing gap;
- Amplitude of spike indicates the size of the particle.

- the “electrical current exclusion” principle
- the “resistance measurement” principle
- the “electrical sensing zone” method

“How to Make It and How to Use It”, University of Kansas, Lawrence, KS August 2019
Electronic System & Output

The pulses produced at the orifice are amplified and displayed on the oscilloscope screen and appear as vertical lines or spikes. The height of an individual pulse spike from the baseline is a measure of relative size of the cell. The threshold control dial enables the user to set a cut off. Only if the pulse exceeds the threshold, it gets counted. In addition to a display of relative cell size the oscilloscope also indicates the threshold control by brightening that portion above the threshold level.

The replacement of electrolyte in the sensing zone by cell results in the momentary change of electrical impedance ($\Delta R$).

$$\Delta R = \frac{4 \rho_m d_p^3}{\pi D_t^4}$$

$d_p$ – diameter of passing cell with diameter, $D_t$ – diameter of the orifice/microchannel, $\rho_m$ – resistivity of the electrolyte.
Aligning Particles Through the Orifice

A particle passes through the orifice and produces a change in the resistance of the fluid-filled channel.

- identical particles flowing through different trajectories experience different electric field strengths and generate different magnitude signals

Better signal reproducibility if particles “aligned”

- sheath flow
- inertial focusing
- dielectrophoresis
- acoustophoresis

“How to Make It and How to Use It”, University of Kansas, Lawrence, KS August 2019
Coulter Counter Advantages

- unaffected by particle optical properties
- unaffected by particle chemistry
- no need for colorimetric/fluorescence
- linear over a wide range of concentration
- operate over a wide size range
- will detect any particle that can displace liquid
- simplicity of design
- low power consumption
- the ability to detect individual particles
Coulter Counters - Applications

- widely adopted
  - Used to count cells/determine cell size (mammalian, bacteria, virus)
  - particle size analysis of almost any powder, suspension or emulsion,
  - Coulter principle is used in quality control of consumer products, i.e., chocolate and beer, paint and toners

  blood cells, colloidal particles, DNA, viruses, antigens and pollen

- made possible medical diagnostic test: the complete blood count (CBC)

Commercial Coulter Counters

Beckman Coulter AcT Coulter

Beckman Coulter Z2 Particle Counter Size Analyzer

Beckman Coulter™ Multisizer™ 3 Coulter Counter®

Handheld particle counter

“How to Make It and How to Use It”, University of Kansas, Lawrence, KS August 2019
Examples of Commercial Miniaturized Coulter Counter Instruments

Scepter Coulter counter tip:
- Outlet electrical contacts
- Coulter counter
- Pre-filter
- Inlet

Moxi Z chip

Micropore
- Pore diameter: 40 µm
- Pore length: 90 µm

Cross-section

"How to Make It and How to Use It", University of Kansas, Lawrence, KS August 2019
Microfluidic Coulter Counters - Constriction Channel Design

How to Make It and How to Use It, University of Kansas, Lawrence, KS August 2019
Electrical Impedance: AC or DC?

• extends the concept of resistance to AC circuits,
• possesses both magnitude and phase, (unlike resistance, which has only magnitude).
• When a circuit is driven with direct current (DC), there is no distinction between impedance and resistance; resistance can be thought of as impedance with zero phase angle.

The ratio of a sinusoidal voltage to a sinusoidal current is called "impedance“ (generalization of Ohm's Law for resistors).

\[ I = \frac{V}{Z} \quad \text{for a pure resistor where} \quad Z=R, \text{preserving the form of the DC Ohm's law.} \]
Polarization Effect in DC Coulter Counters

• Effects of electrode polarization (formation of an electrical double layer at the electrode)
• DC Coulter counters:
  • a large portion of the dc voltage applied to the electrodes is lost across the double layer,
  • only a small portion of the applied voltage appearing across the channel resistance.
• Particle sizes are significantly underestimated in comparison to their actual sizes if the polarization effect is not taken into account!
• To reduce effects of electrode polarization, Ag/AgCl electrodes can be used to enhance the charge transfer across the electrode/electrolyte interface microelectrodes made of gold or platinum, Ag/AgCl, platinum black electrodes, porous platinum black electrodes, gallium–indium

Jagtiani et al. 2011, Journal of Micromechanics and Microengineering, 21, 4, 045036

“How to Make It and How to Use It”, University of Kansas, Lawrence, KS August 2019
• different frequencies (f) can be used
  • high enough frequencies so double layer impedance is negligibly small, *(measurement of impedance pulses requires complex hardware and algorithms for signal processing)*
  • low enough frequencies so impedances caused by channel and stray capacitances are large enough - the main current path is through the channel resistance
  • the channel resistance is dominant between 100 kHz o 10 MHz
**Limitations of Commercial Coulter Counters**

- large sample size required
- only portion of the sample is processed
- difficult to quantitatively recover cells for other assays
- difficult to integrate with other flow-through assays
- expensive

**Microfluidic Coulter Counters**

- inexpensive, polymer microfluidic module with printed electrodes
- low internal volume (~2 µL) for handling small sample aliquots
- easy recovery of cells for subsequent analysis
- easy to integrate with other microfluidic assays

“How to Make It and How to Use It”, University of Kansas, Lawrence, KS August 2019
Fabrication of Micro Coulter Counter (µCC)

microfluidic component (PMMA) + Coverplate (PMMA) patterned with electrodes → bonding → µCC chip
Fabrication of Fluidic Component

CAD design and High Precision Micromilling

Hot embossed chip

PMMA chip

PMMA chip

inlet

outlet

orifice

electrode

electrode

Cross-section of the orifice

Cross-section of the inlet channel

Air

50 µm

Pore sizes: width: 30 µm, length: 40 um, height: 40 um

“How to Make It and How to Use It”, University of Kansas, Lawrence, KS August 2019
Fabrication of Coverplate and Electrodes

How to Make It and How to Use It, University of Kansas, Lawrence, KS August 2019

Milled "grooves" in the coverplate for silver paste

"Grooves" filled with silver paste

Dry electrodes 1 h – overnight at 65°C

Silver/Silver chloride paste/ homemade pen

Carbide 3D desktop milling machine
1/32” milling bit; 5,000 rpm

Drawing AutoCAD

Silver/Silver chloride paste/ homemade pen

Ag/AgCl electrodes

Milled "grooves" in the coverplate for silver paste

5 mm
Four-Point Probe Electrode Configuration

- Four-point measurement method:
  - two pairs of microelectrodes (Ag/AgCl) used to overcome polarization problem
  - setup consists of four equally spaced electrodes.
  - a high impedance current source supplies current through the outer two probes;
  - a voltmeter measures the voltage across the inner two probes to determine the sample resistivity

\[ R = 2\pi s \frac{V}{I} \]
Chip Preparation

Dice the Devices (Bandsaw)

Drill inlet/outlet holes (drill press, d=0.85 mm)

Wash, sonicate and air dry chips

Dry chips (65°C)

5% Micro-90 (detergent), 2 minutes sonication, rinse with DI water

PMMA and PC: few hours to overnight
COC: 1 h or less

“How to Make It and How to Use It”, University of Kansas, Lawrence, KS August 2019
Chip Assembly and Bonding

- Chip and coverplate: UV/Ozone treatment (5 min)
- Align electrodes with fluidic network
- Mount assembly between glass plates, secure with strong clamps
- Thermal bonding in the oven, 15 minutes at 96°C
- Place tubing to outlet/inlet of your device (**PEEK tubing**), secure with epoxy glue

Go and Experiment!
μ-Coulter Counter Instrument

Testing Setup

50 kHz, 6V-14V

Electronics for signal generation and acquisition

NI-DAQ card
Custom-built PCB
electrode connector

LabVIEW code for data collection

Matlab code for data analysis

20 kHz data collection

“How to Make It and How to Use It”, University of Kansas, Lawrence, KS August 2019
Counting Dye Labeled Beads
Matlab for Data Analysis

“How to Make It and How to Use It”, University of Kansas, Lawrence, KS August 2019
Performance of μCoulter Counter

Histogram of cell sizes measured by microscopy

Histogram of μCC signals of RPMI-8226 cells and calibration beads

RPMI 8226 cells
15 µm calibration beads
20 µm calibration beads
Acknowledgments

Dr. Matt L. Hupert

Prof. Soper Research Group
Dr. Matt Jackson
Ms. Swarna Vaidyanathan
CBM² Members

Adams Microfabrication Facility at KU

P20GM103638
ASKING QUESTIONS

SMALL QUESTIONS

LEAD TO SMALL DISCOVERIES.

BIGGER QUESTIONS

LEAD TO BIGGER DISCOVERIES.

SOME QUESTIONS

ONLY REVEAL DEEPER MYSTERIES.

EVEN IF YOU KNOW WHAT QUESTION TO ASK

THE ANSWER MAY SURPRISE YOU.

GRANT SMIDER
Microfluidic Impedance Sensors – not a standard Coulter Counter

- particle/cell flows between a pair of electrodes
- the electric field between these two electrodes is disrupted, resulting in a current change (measured at point A)
- the current measured at this position corresponds to the impedance of cell and its suspending medium.
- to determine impedance of medium, the current at point B is also acquired simultaneously and the impedance of cell can be acquired from the difference between current at point A and at point C

Typically, the setup consists of pre-amplifier, lock-in amplifier and data acquisition system. The excitation signal is supplied to excitation electrode by function generator or lock-in amplifier, and sensing electrodes are connected to bridge circuit or trans-impedance amplifiers to measure current response of system. The amplifiers' output is connected to lock-in amplifier to demodulate current signal at excitation frequency. The data are sent to data acquisition system for post processing.

“How to Make It and How to Use It”, University of Kansas, Lawrence, KS August 2019