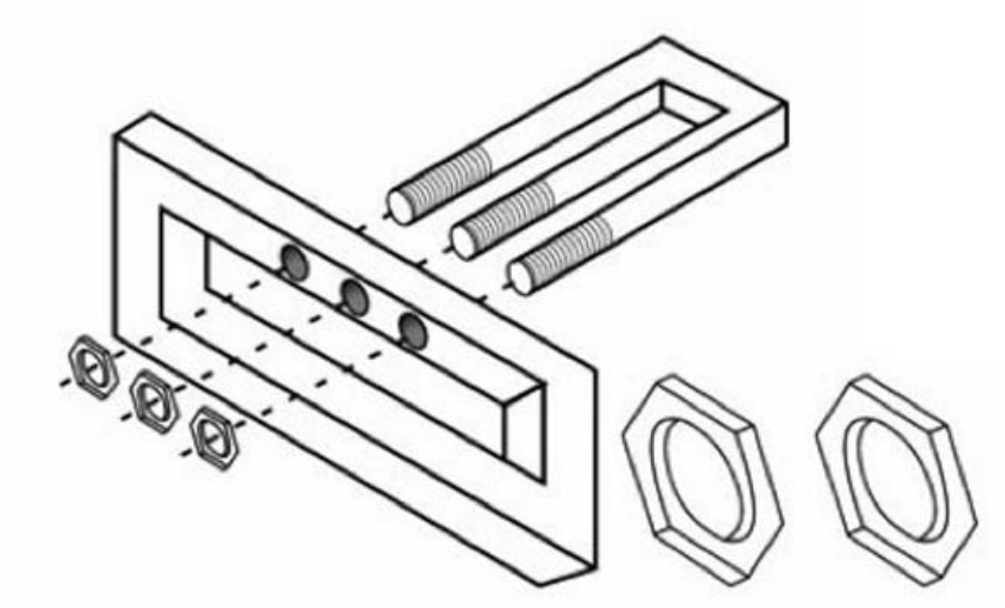
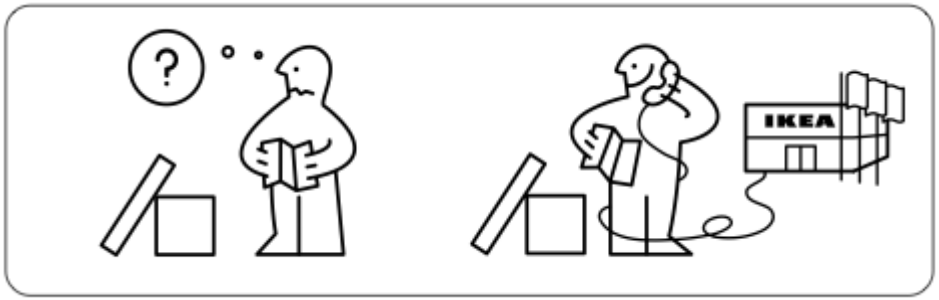
TECHNOLOGIES FOR CLINICAL CELL SORTING

- SAMPLE THROUGHPUT
- STERILITY & SAFETY
- EASE-OF-USE

SOP for Droplet Sorter





TECHNOLOGIES TO ADDRESS:

- **SAMPLE THROUGHPUT**
- **STERILITY & SAFETY**
- **EASE-OF-USE**

SAMPLE THROUGHPUT IN CLINICAL TRIALS

- **Starting material: 400 – 800 million cells**
- **Sort time: 5 – 16 hours**

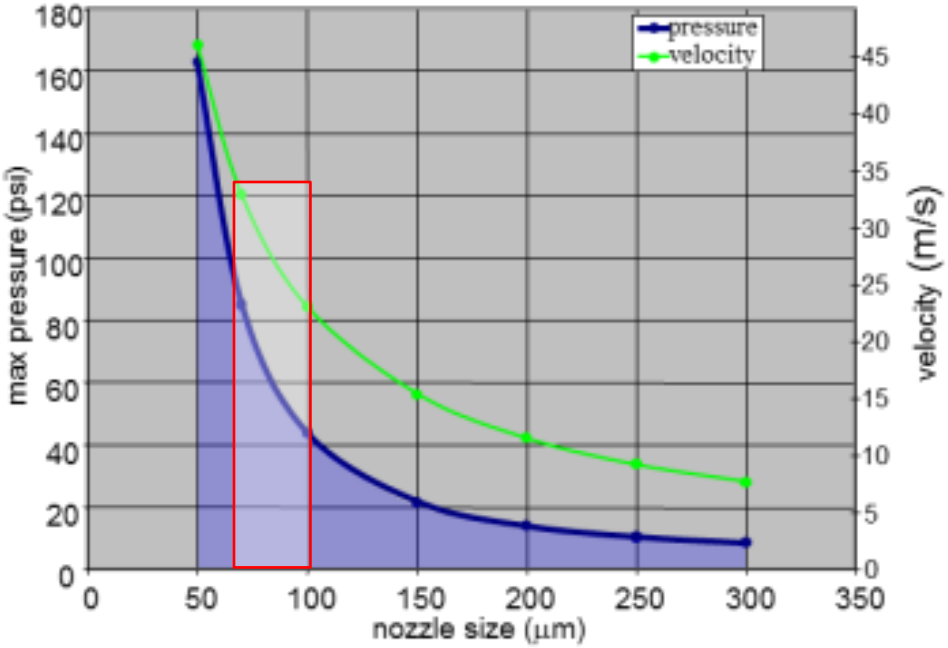
7,000 – 20,000 Ev/sec

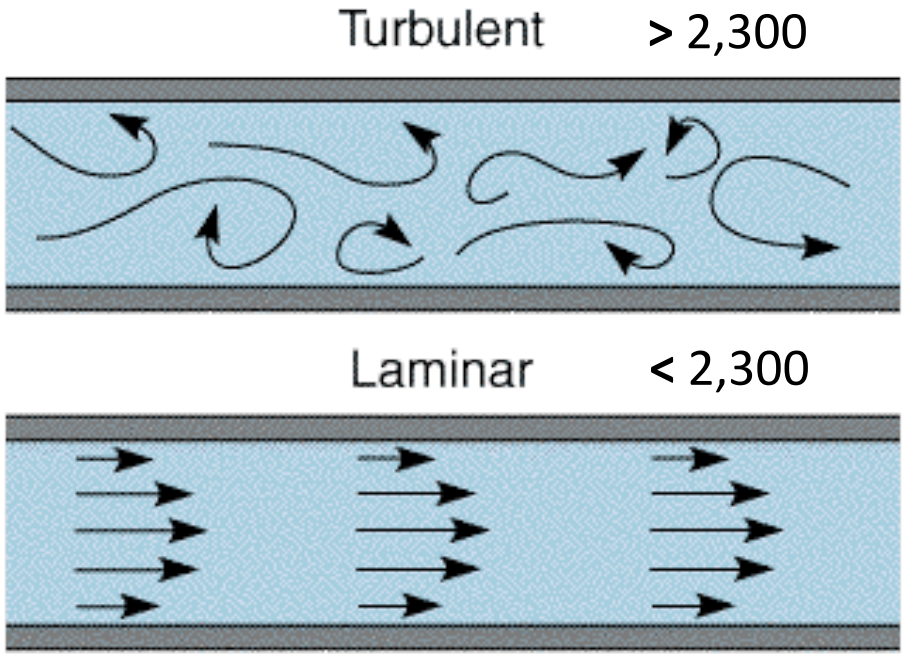
SAMPLE THROUGHPUT WITH HIGH-SPEED CELL SORTERS

50 million per mL **100 million per mL** **200 million per mL**

detection rate	12000 Ev/sec		24000 Ev/sec		48000 Ev/sec	
	purity	**sort efficiency**	**purity**	**sort efficiency**	**purity**	**sort efficiency**
droplet sorter A	83%	85%	74%	70%	-	-
droplet sorter B	98%	76%	98%	69%	-	-

THROUGHPUT LIMITATIONS

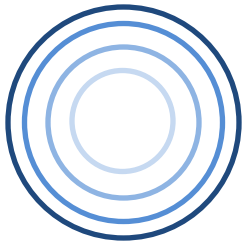




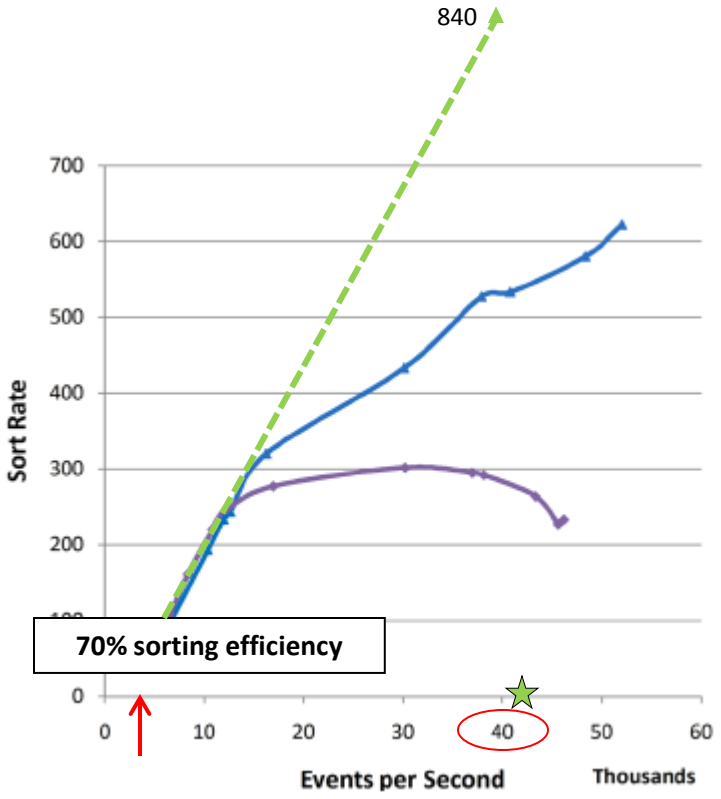
$$\text{Reynolds Number} = \frac{d.\rho.V}{\eta}$$

$V = (\Delta P/\rho)^{1/2}$

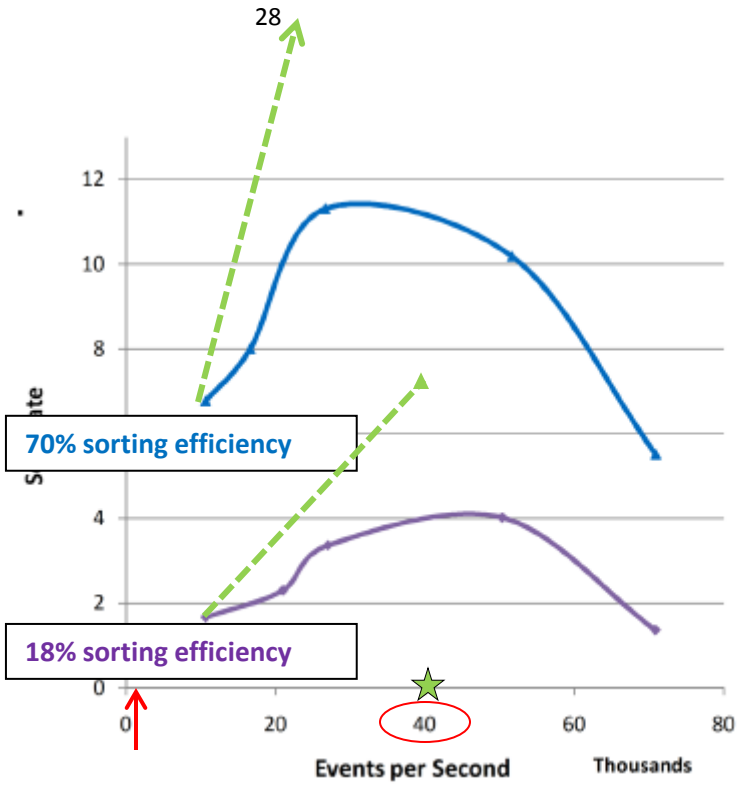
internal diameter (d)
fluid density (ρ)
velocity (V)
viscosity (η)



ULTRA HIGH-SPEED SORTING



3% target population



0.1% target population

Aria II

MoFlo XDP

Parallel performance

Comparison of Sorting Capabilities of Beckman Coulter MoFlo™ XDP and Becton Dickinson FACSAria™ I and II. Ross C. et al. BeckmanCoulter 2009 White Paper

PARALLEL SORTING IN CLINICAL TRIALS

- **Starting material: 400 – 800 million cells**
- **Sort time using 3–4 Sorters: 1.5 – 5 hours**

- **or run 2.5 – 5 billion cells in 5 – 16 hours**

ULTRA HIGH-SPEED SORTING: PARALLEL SORTING

TECHNOLOGIES TO ADDRESS:

- SAMPLE THROUGHPUT
- **STERILITY & SAFETY**
- EASE-OF-USE

STERILITY & SAFETY IN CLINICAL TRIALS

- **Sterile and Enclosed environment**
- **Single–Use components**
- **Pre–assembled units**

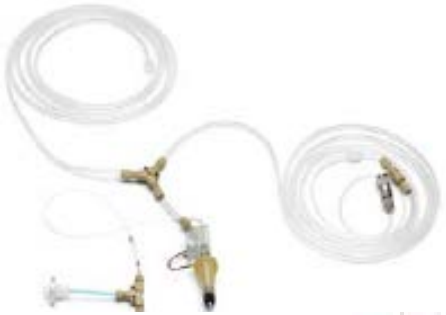
- **Operator not exposed to hazardous material**

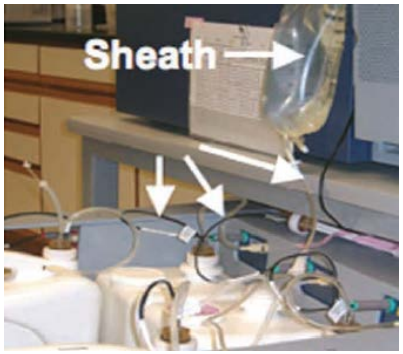
STERILITY AND DROPLET SORTERS

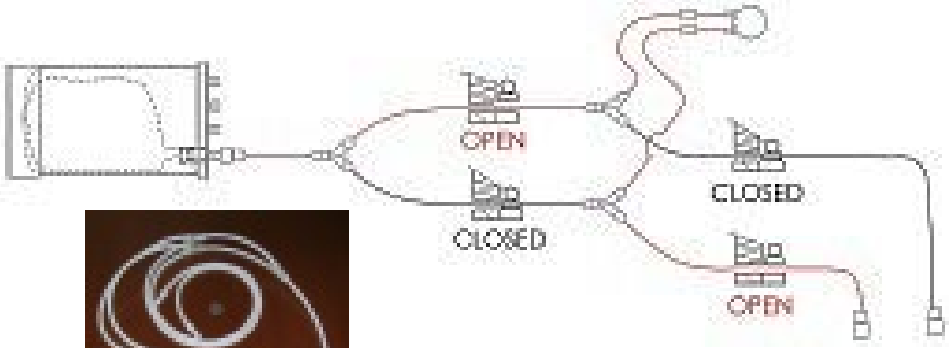
Product Contact Parameter	R&R	SIP	Other	Comments
sample container	★			sterile disposable
sample path	★			tubing and o-rings replaced with kit components
flow cell / nozzle holder		★		6% peroxide or ethanol
nozzle tip		★		6% peroxide or ethanol
product container	★			sterile disposable
sheath tank	★			cleaned with disinfectant, rinsed, autoclaved
sheath delivery path tubing		★		6% peroxide or ethanol
sheath tank filter	★			filter capsule replaced with kit component
fittings & couplings	★			fittings and couplings replaced with kit components
sheath fluid	★			sterile commercial product
system pressure (nitrogen)			★	multiple stage filters, final filter is also a component of the aseptic sorting kit
environmental air			★	HEPA filtered, class 1000 air handling

THANKS to Michael Reitsma

STERILE, DISPOSABLE SYSTEMS FOR DROPLET SORTERS







THANKS to Jeff Haug, Chris Groves and Darren Hickerson

NIH POLICY(?) FOR CELL SORTERS

- Personal Protective Equipment
- Cell sorter in separate space
  - or in Class II BSC
- Method for evacuation of aerosols
- Validation of aerosol evacuation method

BIOSAFETY AND DROPLET SORTERS

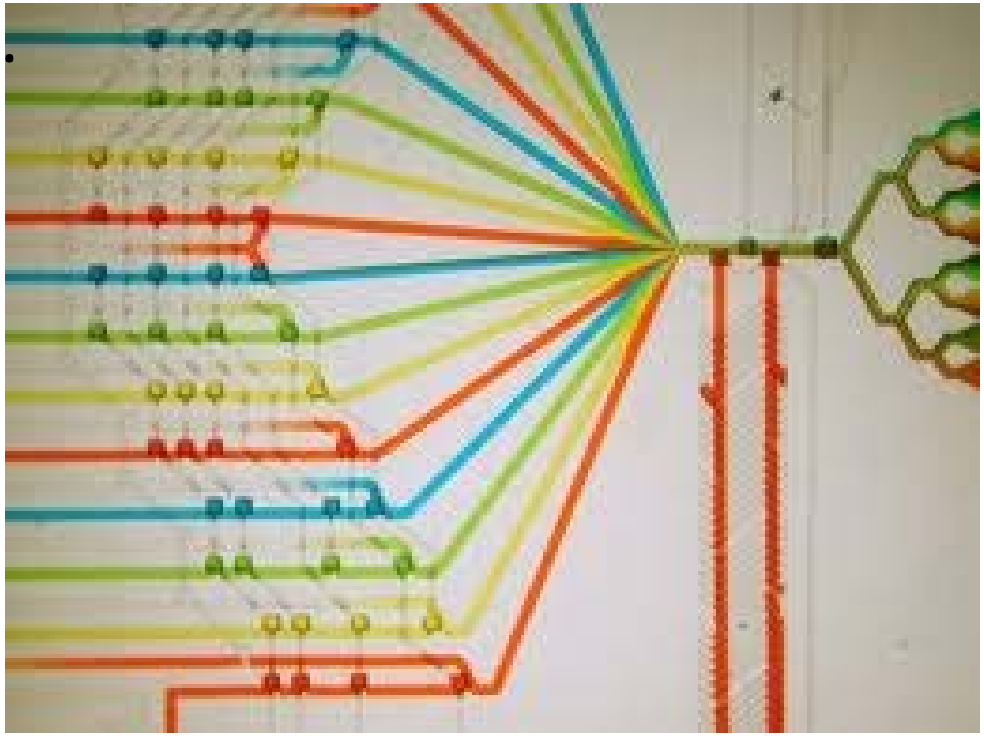
THANKS to Peter Lopez, Tricia Rogers, Chris Groves and Karen Clise-Dwyer

EMERGING TECHNOLOGIES TO ADDRESS:

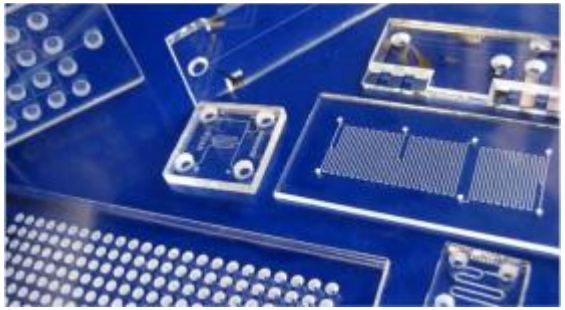
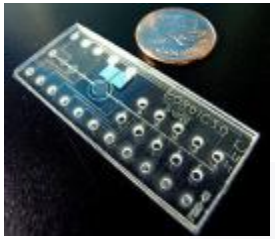
- SAMPLE THROUGHPUT
- STERILITY & SAFETY
- EASE-OF-USE

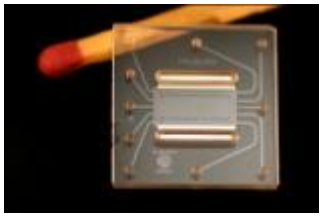
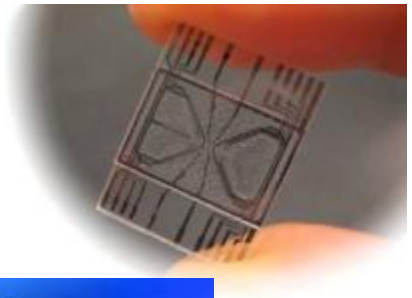
NO DROPLETS: MICROFLUIDICS …

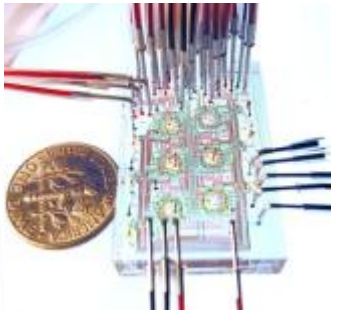
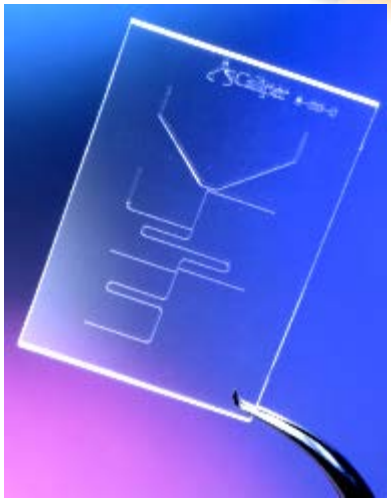
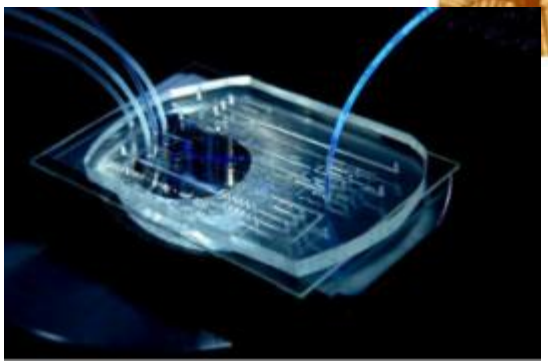
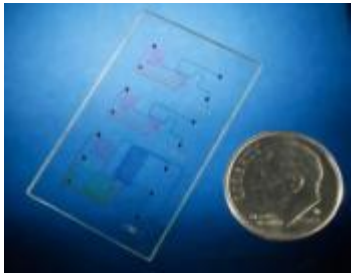
- …” deals with the behavior, precise control and manipulation of fluids that are geometrically constrained to a small, typically sub-millimeter, scale.”



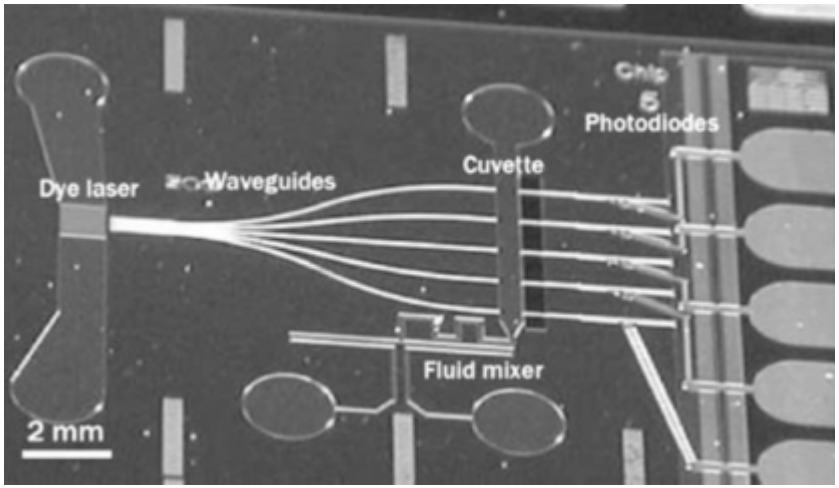
CHIPS

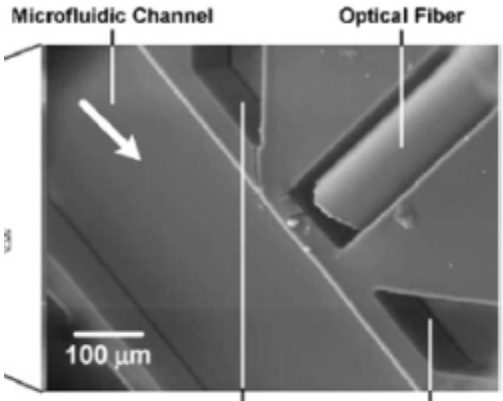




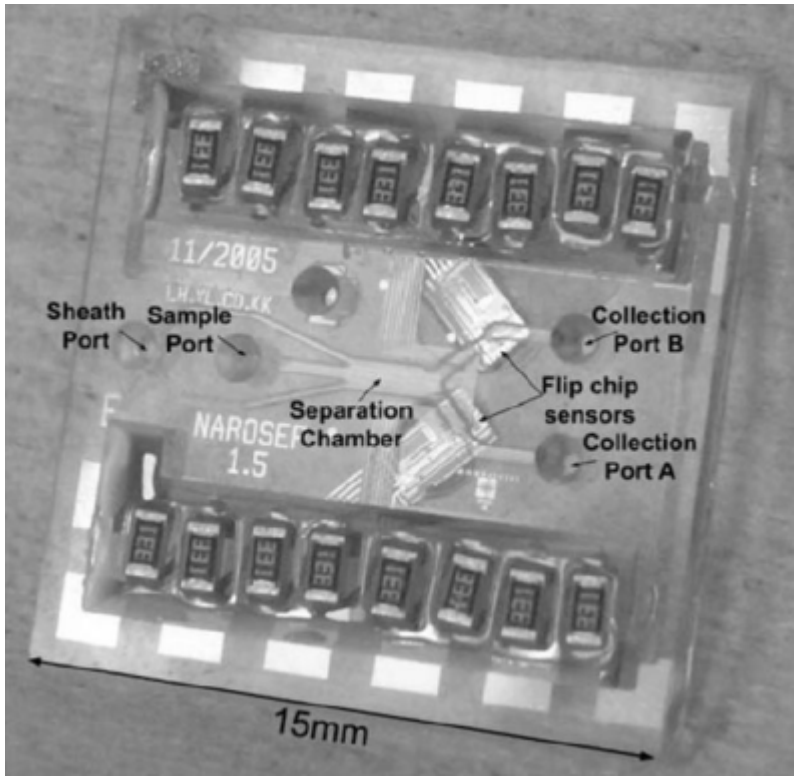
MICROFLOW CYTOMETERS



Balslev S., et al. Lab Chip 2006;6:213–217



Tung Y-C, et al. Sensors Actuators B 2004;356–367



Hartley L, et al. IEEE Trans Circuits Syst I 2007; 54:99–110

TYPICAL ADVANTAGES OF MICROFLUIDICS

- Low-cost
- Point-of-care
- Small amounts of reagent
- Enclosed (biosafety)

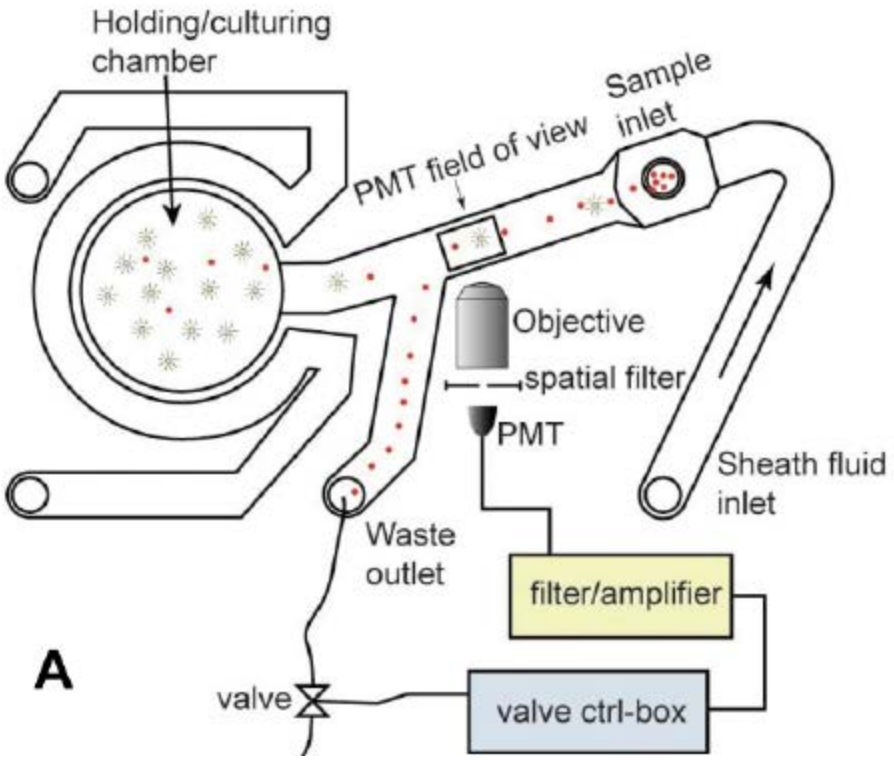
and also … BIOCOMPATIBILITY

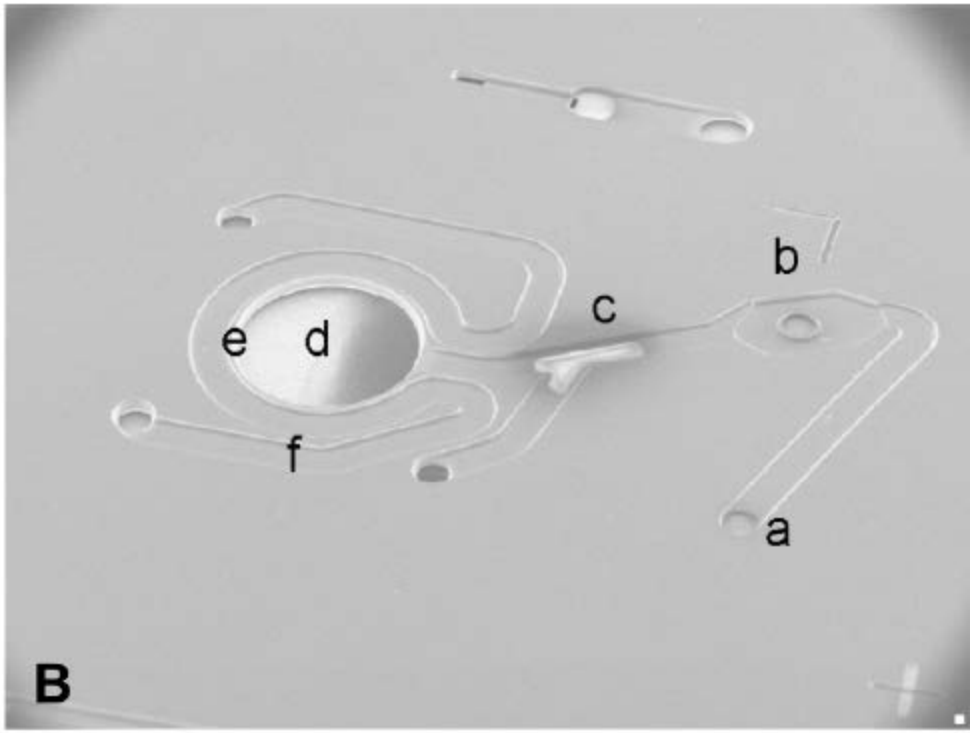
Cell culture flask	Microfluidics
Small surface to volume ratio	**Large surface to volume ratio**
Static environment (build up waste)	**Constant renewal of media (removal of waste)**
Turbulent flow of fluid (or static)	**Laminar flow of fluid**
Set pressure	**Variable** (controllable) **pressure**
Large media consumption	Small media consumption
Slow temperature changes	**Rapid temperature changes**
No shear stress	Control of shear stress
No integration to instrumentation	Integration to instrumentation

BOLD: most In vivo conditions

Abigail Webster et al. J. Chem. Technol. Biotechnol. (2010)

SORT AND CULTURE

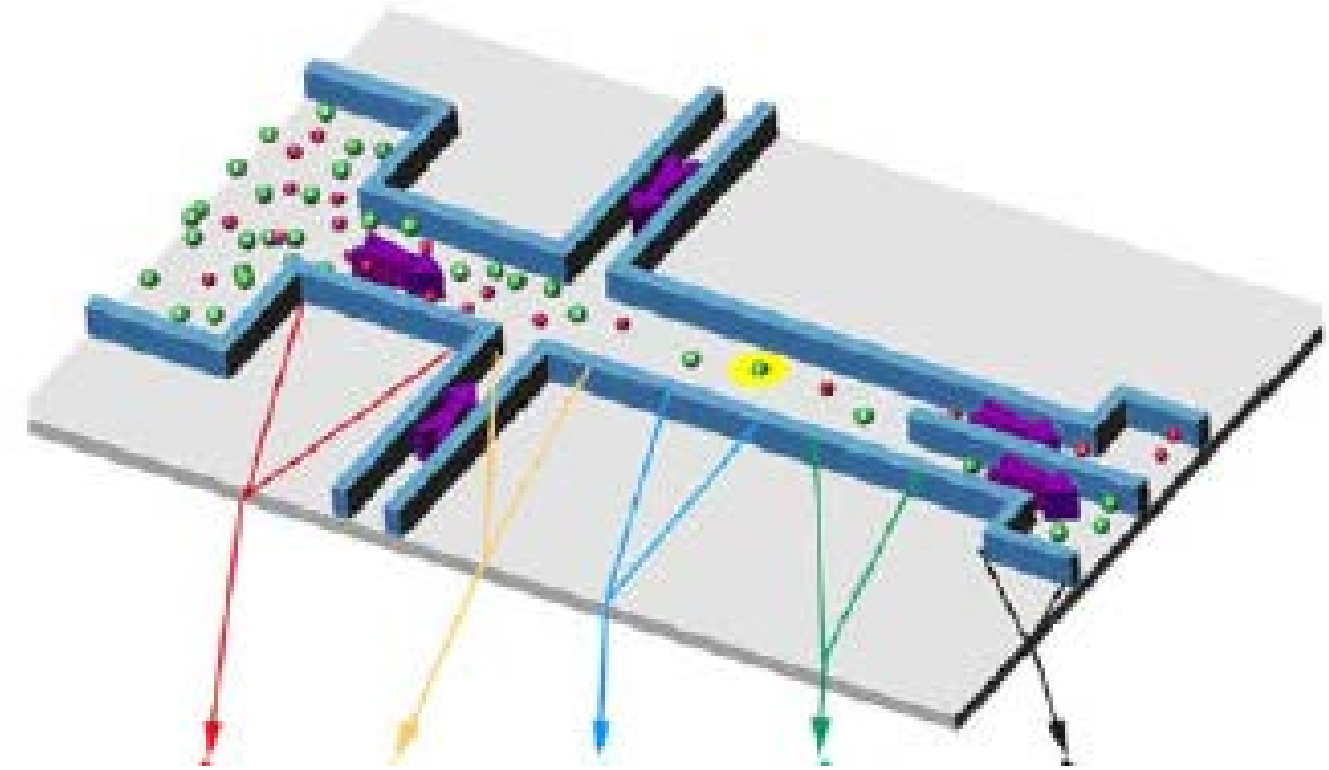




12,000 ev/sec (sort 0.3 Ev/sec)
0.0024% target population enriched to 0.24%
Sample concentration ~100K cells/mL

Wolff A. et al. Lab Chip 2003 ;3: 3-27 .

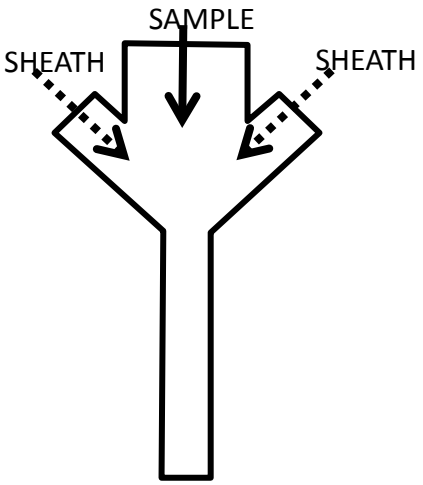
5 STEPS IN SORTING

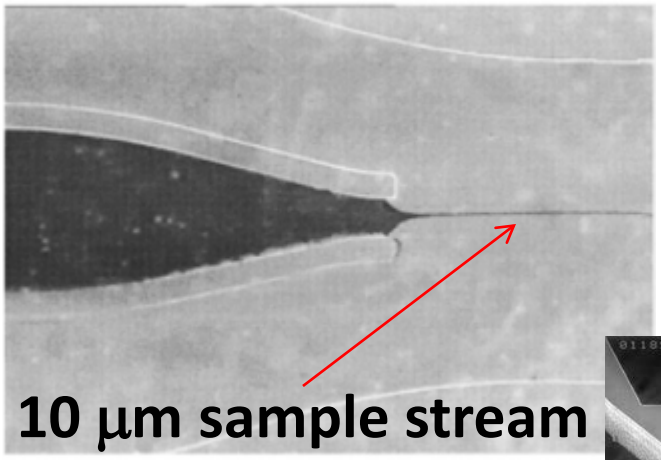


Transportation Focusing Detection Separation Collection

CORE FORMATION – 2D

- Jacobson SC, Ramsey JM. Anal Chem 1997;69:3212–3217
- Most widely used sheathing system in microcytometry
- Suitable for microscopy and video imaging
- Illumination and detection efficiency not uniform

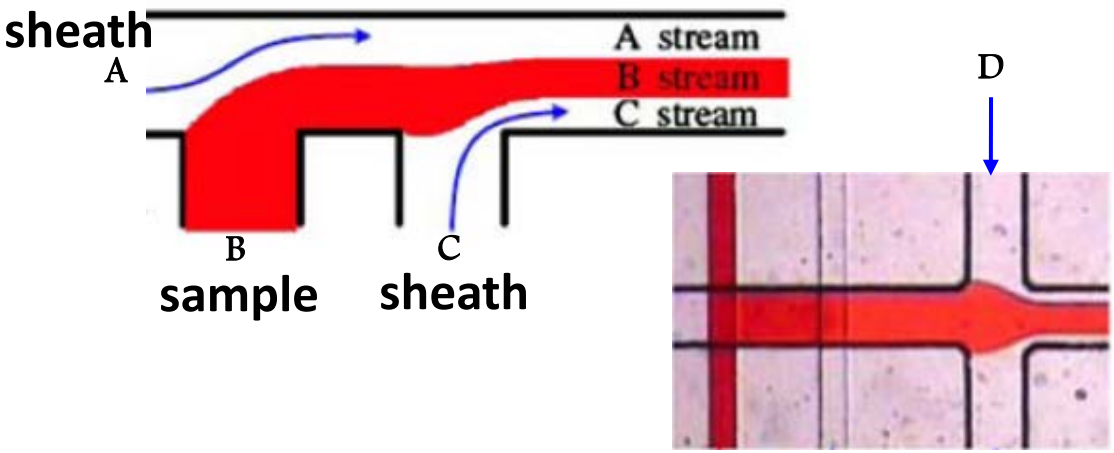


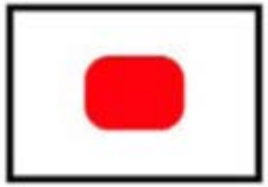


Lee G.B. et al. Trans. ASME I 2001; 123, 672-679.

CORE FORMATION -3D

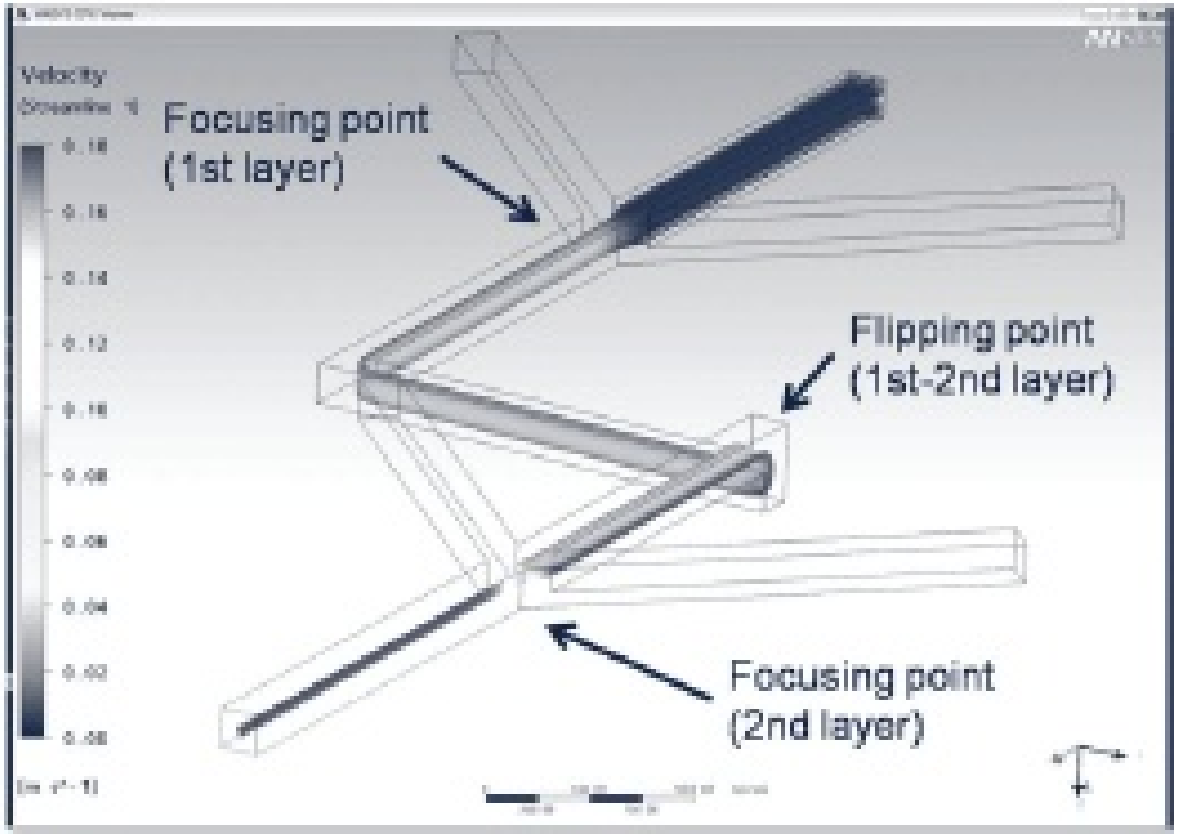
- Required for ‘flow cytometric’ detection
- Complex fabrication process
- 4-6 sheath inlets





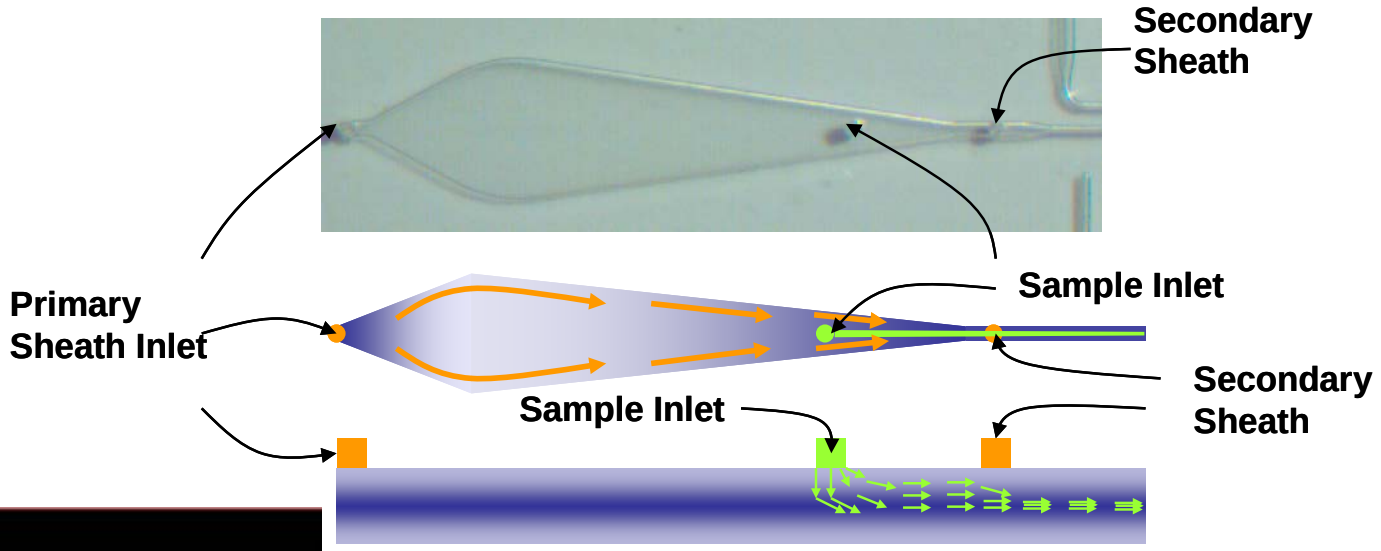
Chang C.C. et al. Micromechanics and Microengineering 2007;17: 1479-1486.

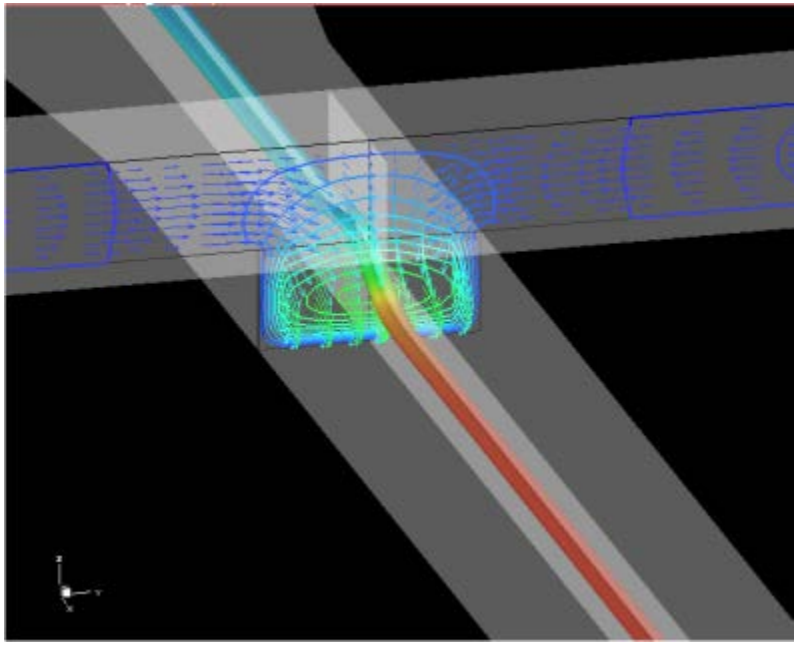
CORE FORMATION -3D

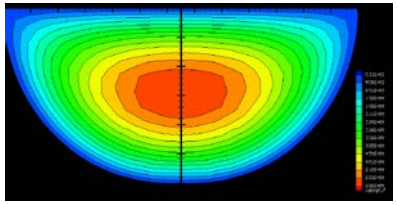


Shinado M. et al. Cytometry PartA 2008; 114 (abstract).

CORE FORMATION -3D

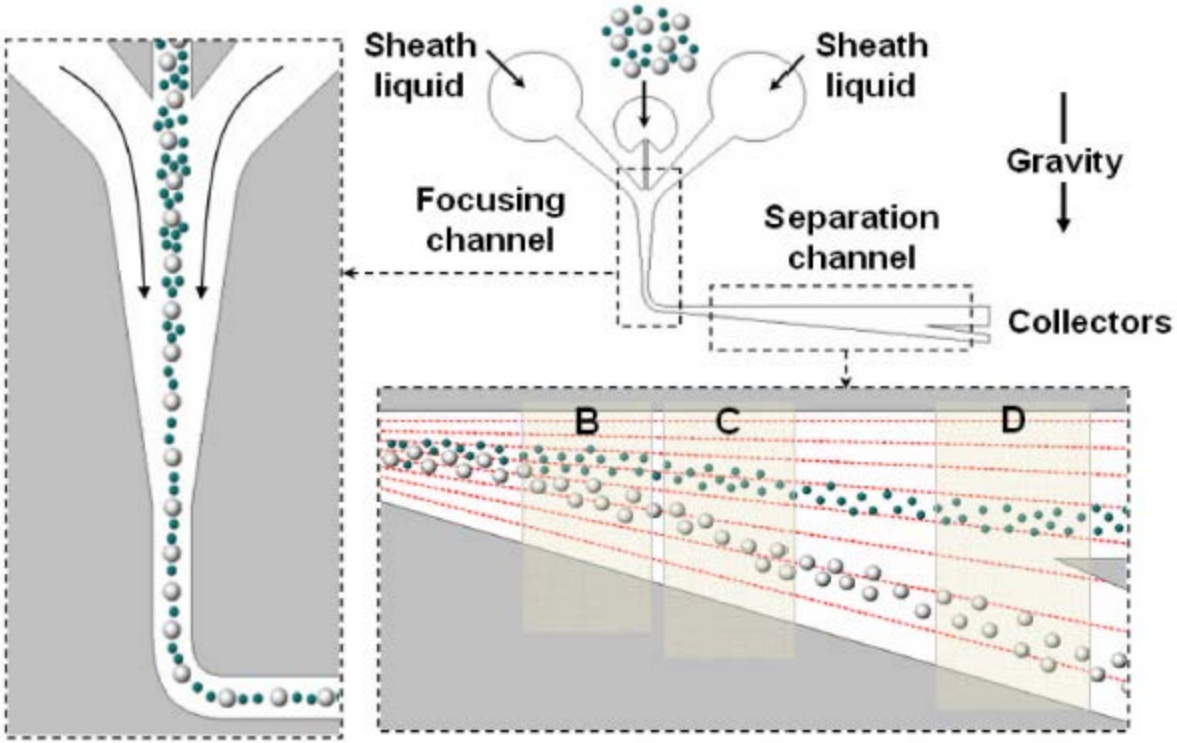






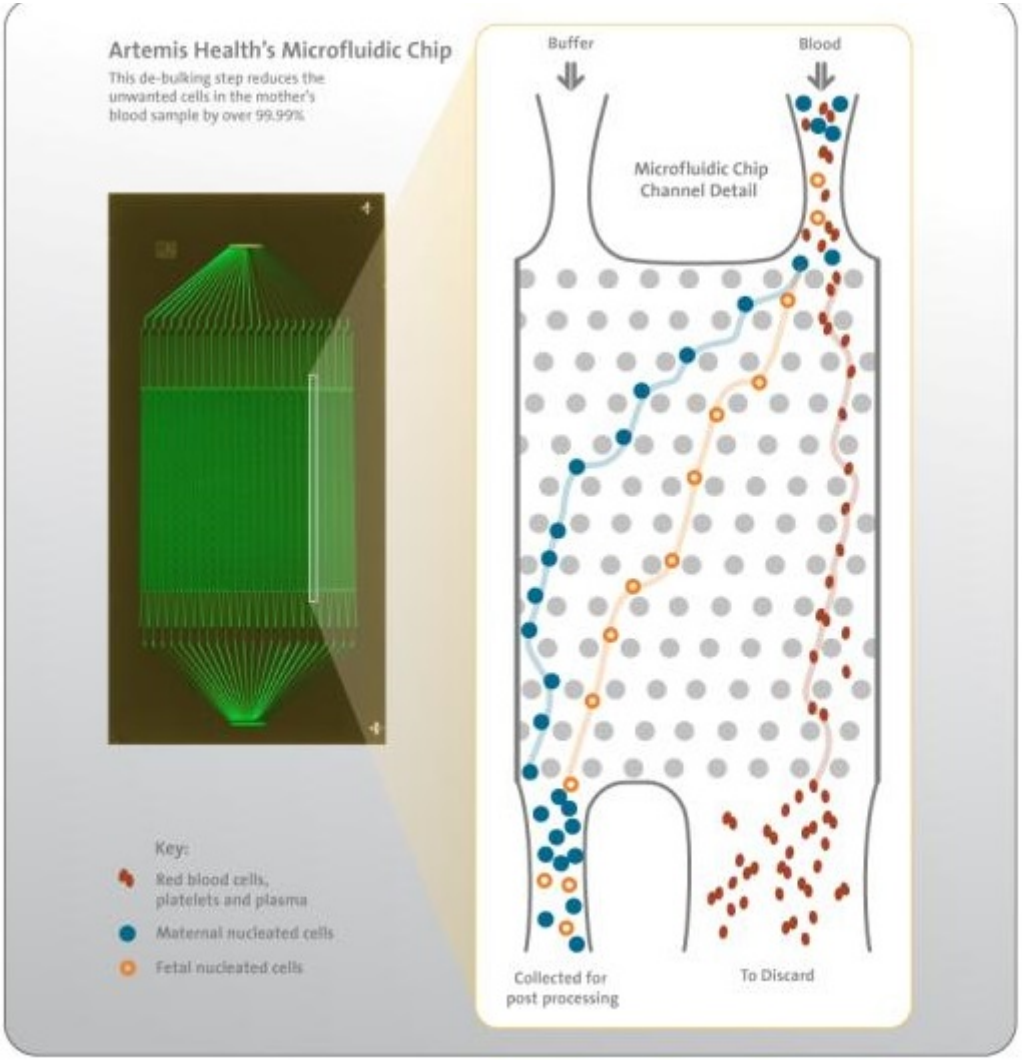
Gilbert JR, Böhm S, Deshpande M, 1998

SEPARATION BY GRAVITY



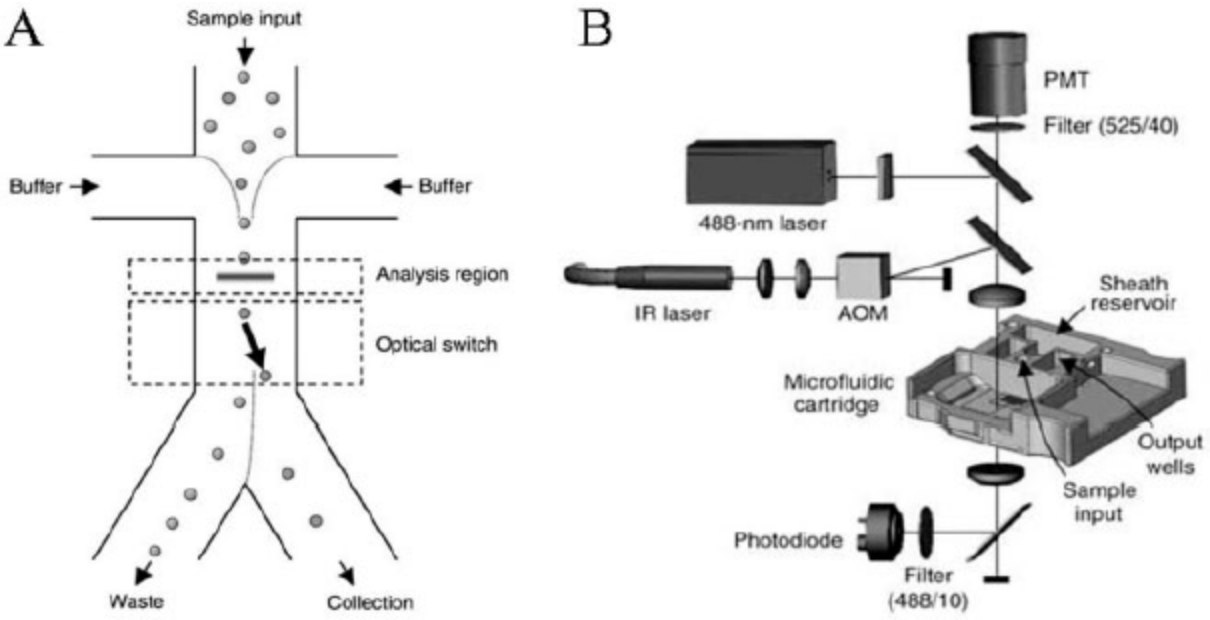
Huh D et al. Anal Chem. 2007 ;79: 1369-1376 .

SEPARATION BY SIZE



ARTEMIS

OPTICAL SWITCH

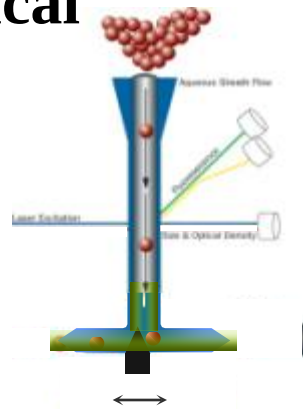


1,000 – 280,000 ev/hour (0.3 – 78 ev/sec)

Wang MM. et al. Nat. Biotechnol. 2005 ;23: 83-87 .

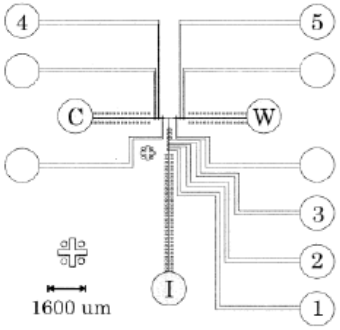
FLUIDIC SWITCHES

Mechanical

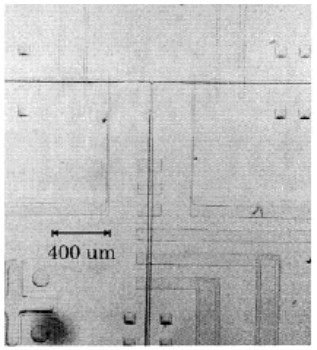


Fluidic switch

Kamentsky and Melamed (1967), Science



(C) Microfabricated Cell Sorter



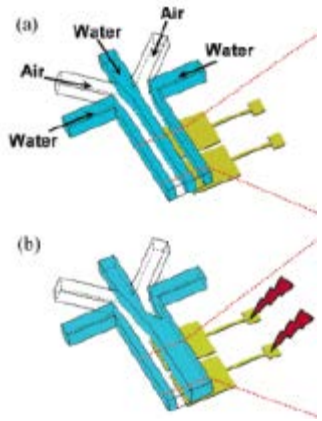
(D) Actual RTV device

Fu et al. (2002), Anal.Chem.; 74, 2451-2457

10 – 5 ms switch time

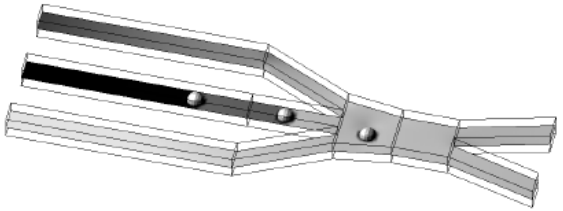
(100 – 200 sorts/sec)

Electrokinetic



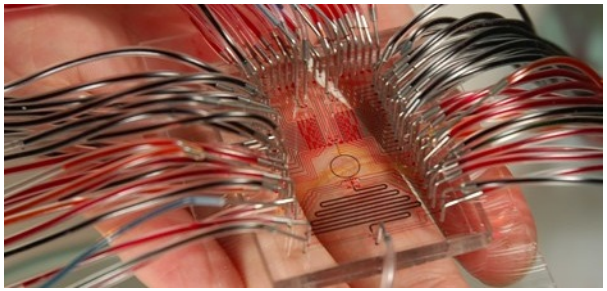
Huh et al. (2003), J. Am. Chem. Soc.; 125, 14678 - 14679

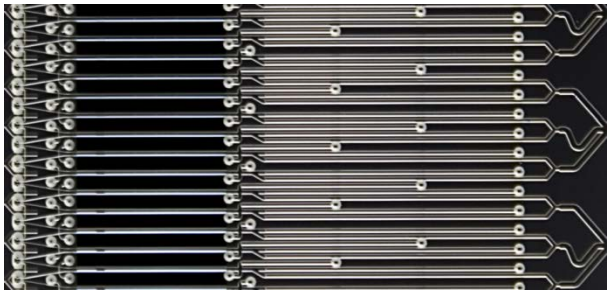
Pressure driven

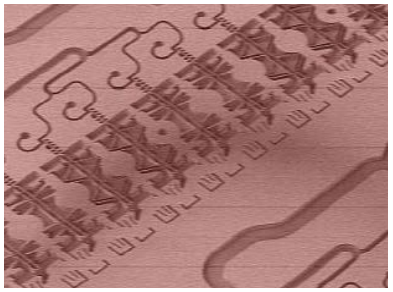


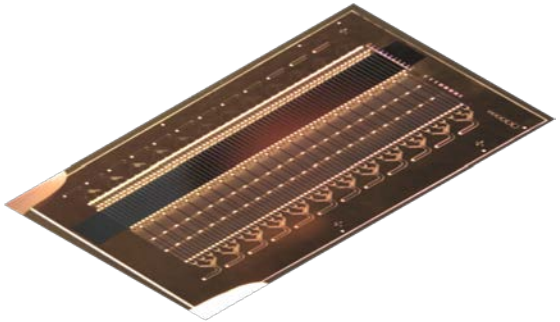
Chen et al. , Transducers, Boston 2003

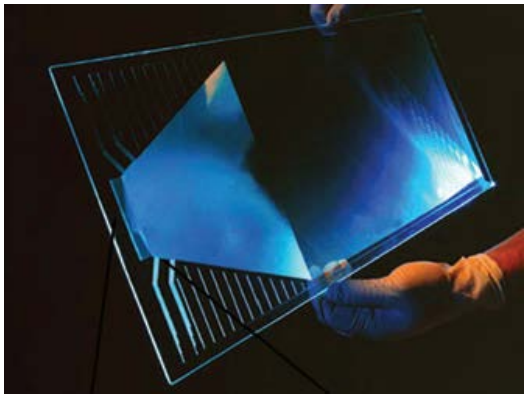
PARALLEL MICROFLUIDIC ARCHITECTURE









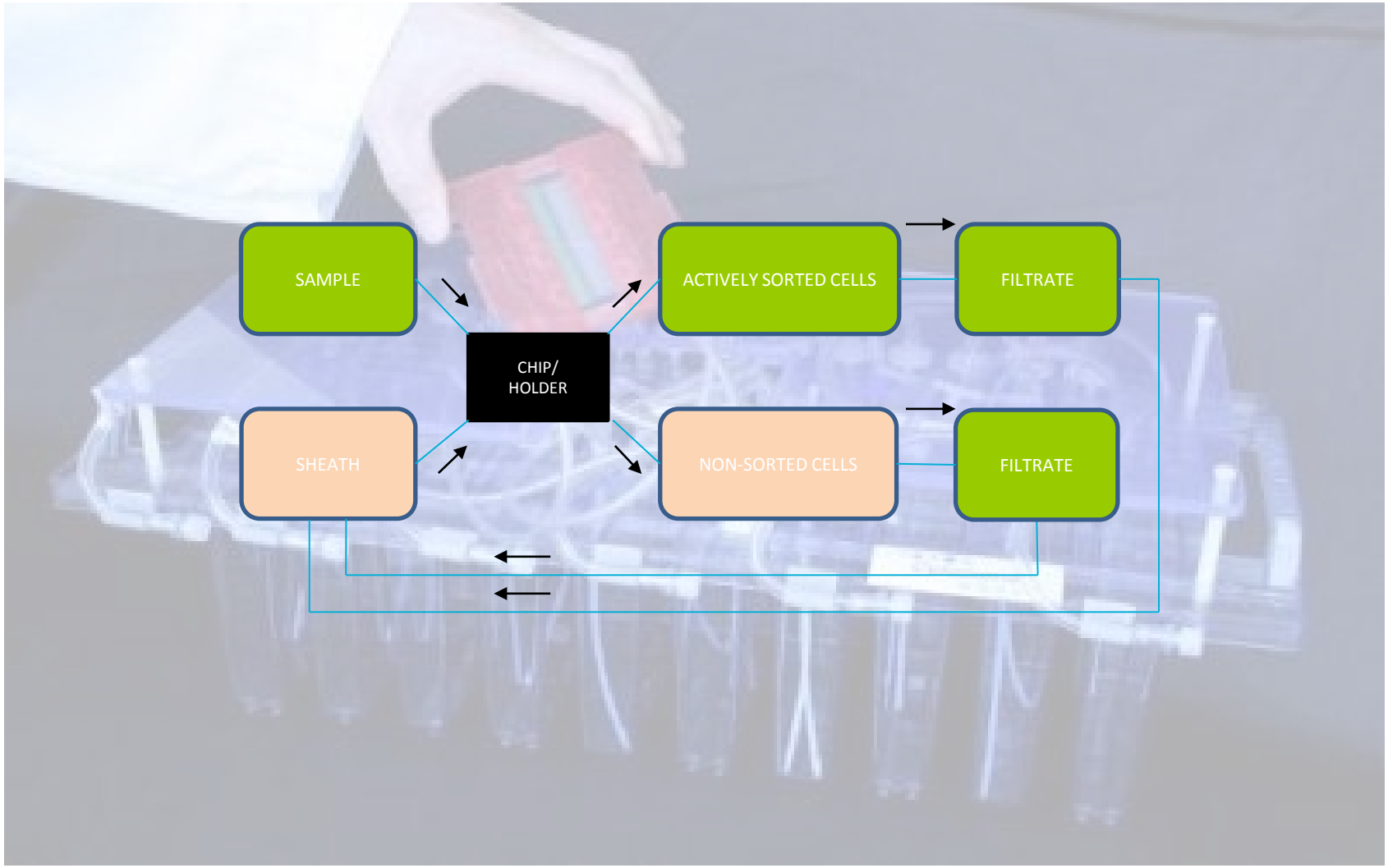


- Fastest fluidic switch instruments operate at 2,000 Ev/sec/channel

- Parallel architectures

  4 - 384 channels

MICRO- AND MACROFLUIDICS



STERILITY & SAFETY

- **Entire fluidic system in cartridge**
- **Single–Use, Sterile, Enclosed**

- **Include pre– and post– sort sample processing steps**

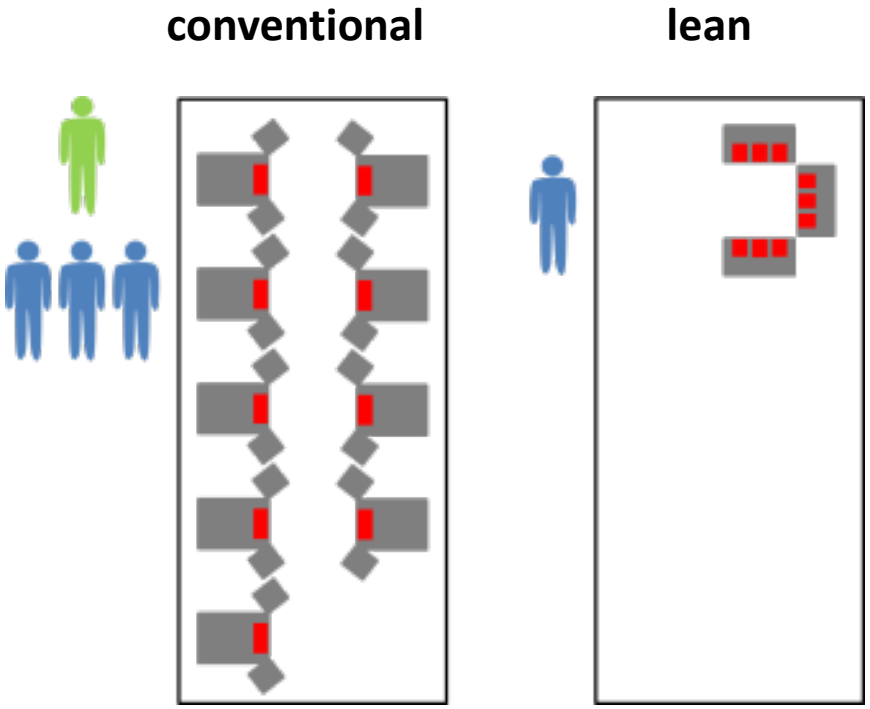
APPROACHES TO ADDRESS:

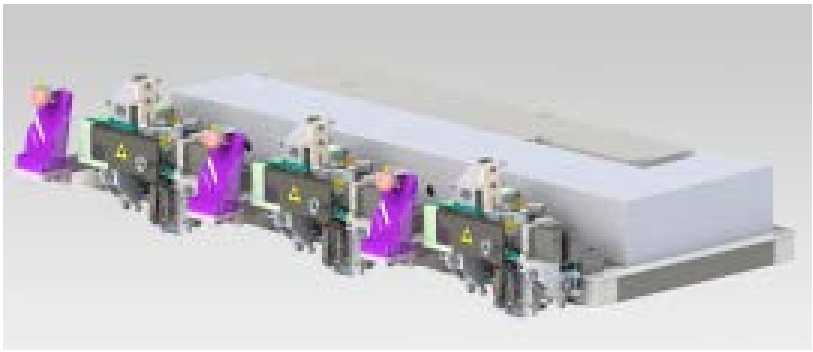
- SAMPLE THROUGHPUT
- STERILITY & SAFETY
- **EASE-OF-USE**

EASY-OF-USE

- Lean process
- Low risk of operator error
- Standard Operating Procedures

LEAN PROCESS





- **3-FOLD RETURN ON CAPTIAL EQUIPMENT**
- **2.5-FOLD RETURN ON FACILITY COSTS**
- **3-FOLD RETURN ON LABOR**

LIMITING OPERATOR ERROR

- Restricted range of applications
  - ✓ Establish specific instrument settings
  - ✓ Eliminate or lock unnecessary features
  - ✓ Simple, unambiguous analysis/sort template
- Process monitoring
  - ✓ Droplet formation
  - ✓ Laser/stream alignment

Digiusto DI and Cooper L. Preparing clinical grade Ag-specific T cells for adaptive immunotherapy trials. Cytotherapy 2007; 9:613-629.

SOP FOR CLINICAL CELL SORTER

Cytometry Part A 67A:76–85 (2005)

Ultra High-Speed Sorting

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Received 10 March 2005; Revision Received 5 April 2005; Accepted 7 April 2005

Background: Cell sorting has a history dating back approximately 40 years. The main limitation has been that, although flow cytometry is a science, cell sorting has been an art during most of this time. Recent advances in assisting technologies have helped to decrease the amount of expertise necessary to perform sorting.
Methods: Droplet-based sorting is based on a controlled disturbance of a jet stream dependent on surface tension. Sorting yield and purity are highly dependent on stable jet break-off position. System pressures and orifice diameters dictate the number of droplets per second, which is the sort rate limiting step because modern electronics can more than handle the higher cell signal processing rates.
Results: Cell sorting still requires considerable expertise. Complex multicolor sorting also requires new and more sophisticated sort decisions, especially when cell subpopulations are rare and need to be extracted from background. High-speed sorting continues to pose major problems in terms of biosafety due to the aerosols generated.
Conclusions: Cell sorting has become more stable and predictable and requires less expertise to operate. However, the problems of aerosol containment continue to make droplet-based cell sorting problematical. Fluid physics and cell viability restraints pose practical limits for high-speed sorting that have almost been reached. Over the next 5 years there may be advances in fluidic switching sorting in lab-on-a-chip microfluidic systems that could not only solve the aerosol and viability problems but also make ultra high-speed sorting possible and practical through massively parallel and exponential staging microfluidic architectures. © 2005 International Society for Analytical Cytology

Key terms: cell sorting; ultra high speed; flow cytometry

CLINICAL CELL SORTING IS ENABLED THROUGH:

- MICROFLUIDIC CHIPS WITH PARALLEL ARCHITECTURES

- APPLICATION- & ENVIRONMENT-SPECIFIC INSTRUMENT DESIGNS