

Dr. Olmsted's Testimony



Peter Olmsted
10X Expert Witness

A. I've explained that, in droplet systems, the droplets do not -- no. They've -- they've come -- the droplets have been -- been formed, and they don't necessarily contact the walls of the container.

Whereas, in segmented flows, the segmented flows are contained within the full width of the container.

Q. And from a flow perspective, do you consider that to be fundamentally different?

A. I do.

Olmsted Dep. Tr. (May 13, 2016) at 94:13-95:2

Strong Recognition of Dr. Ismagilov

Top 100 Chemists, 2000-2010 - ScienceWatch.com - Thomson Reuters

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On February 10, 2011, Thomson Reuters released data identifying the world's top 100 chemists over the past 11 years as ranked by the impact of their published research.

The top 100 is intended to celebrate the achievements of chemists who achieved the highest citation impact scores for chemistry papers (articles and reviews) published since January 2000. Thomson Reuters published the table in support of the International Year of Chemistry.

TOP 100 CHEMISTS, 2000-2010, Ranked by Citation Impact (among those with 50 or more papers)

Rank	Institution	Papers	Citations	Impact
1	Charles M. LEBER Harvard University	74	17,778	240.22
2	Ornar M. YAGHI University of California Los Angeles	90	16,870	220.76
3	Michael CHESTNUT Arizona State University	73	12,910	176.80
4	K. Barry SHARPLESS Scripps Research Institute	60	9,754	162.57
5	A. Paul ALIVISATOS University of California Berkeley	93	14,586	156.87
6	Richard E. SMALLEY Formerly Rice University	60	9,217	153.82
7	Hongjie DAI Stanford University	88	12,768	145.09
8	Xiangang PENG University of Arkansas	59	8,540	144.55

Science Watch Inc [5/14/2015 10:17:41 AM]


PLAINTIFFS' TRIAL EXHIBIT PTX526 CA No. 15-151464

TOP 100 CHEMISTS, 2000-2010

SPECIAL REPORT ON HIGH-IMPACT CHEMISTS

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Fast Moving Fronts
Research Front Maps



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Top 100 Chemists, 2000-2010, Ranked by Citation Impact (among those with 50 or more papers)				
Rank	Institution	Papers	Citations	Impact
82	Rustem F. ISMAGILOV University of Chicago	59	3,437	58.25

PTX526-001, -005

Anal. Chem. 2007, 79, 6682–6692

Monpichar Srisa-Art,[†] Andrew J. deMello,^{*,†} and Joshua B. Edel^{*,†,‡}

Department of Chemistry and Institute of Biomedical Engineering, Imperial College London, South Kensington, London, SW7 2BZ, United Kingdom

The online characterization and detection of individual droplets at high speeds, low analyte concentrations, and perfect detection efficiencies is a significant challenge and has been the subject of intense research efforts in labs to high-throughput chemistry and biology. Herein, we describe the integration of confocal fluorescence spectroscopy as a high-efficiency detection method for droplet detection and characterization. This approach allows for rapid, random mixing, rapid detection, as well as low detection limits have been addressed with the approach described here. We demonstrate a high-throughput, droplet-based platform. Using such a system, droplet size, droplet shape, droplet formation frequencies, and droplet composition can be determined. This approach is applicable to filamentous structures, taking advantage of this approach, we demonstrate a high-throughput biological assay based on the detection of a specific protein in a droplet. By attaching a FRET donor (Alexa Fluor 488) to streptavidin and labeling a FRET acceptor (Alexa Fluor 647) on one DNA strand and biotin on the complementary strand, we demonstrate the formation of a DNA droplet, due to streptavidin–biotin binding, resulting in FRET. Fluorescence bursts of the donor and acceptor in each droplet are detected by a high-speed confocal microscope with avalanche photodiode detectors operating in single photon counting mode. Binding assays were investigated and the binding of streptavidin to biotin was quantified. Binding curves fit perfectly to Hill–Wang models, and the binding ratio between streptavidin and biotin was evaluated and found to be in agreement with the biotin concentration. The detection of specific proteins in a FRET pair was also investigated from the binding results. Efficiency results show that this detection system can

In recent years, microfluidic systems have been successfully used to generate multiphase flows in a variety of formats. Of particular note are those that exploit flow instabilities between immiscible fluids to generate suspended droplets.¹ In simple

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a.demicheli@imperial.ac.uk, joshua.edel@imperial.ac.uk.

(1) Song, H.; Tice, J. D.; Ismagilov, R. F. *Angew. Chem., Int. Ed.* 2003, 42, 7636–7639.

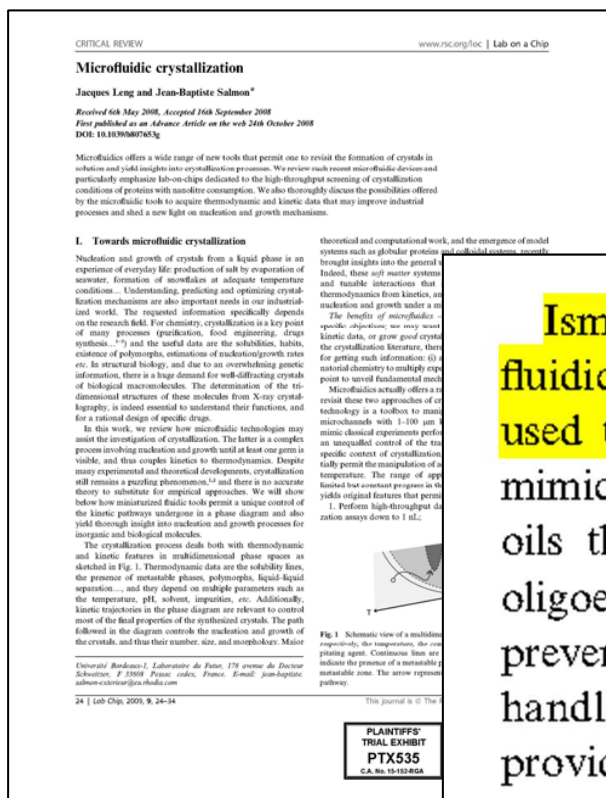
terms, droplets can be made to spontaneously form when luminal streams of aqueous reagents are injected into an immiscible carrier fluid. The formed droplets define picoliter volumes, and because each droplet is isolated from both channel surfaces and other droplets, each acts as an individual reaction vessel.¹ Importantly, variation of the cross-sectional dimensions of the microfluidic system and

exquisite co-
ous phase, il-
trations of re-
studies have
systems to p-
droplet-base
enzyme as-
tion,⁷⁻⁹ nan-
synthesis of
Droplet-base
ment in whil
fluid volum-
elegant ex-
ismaglow ar-
reaction kin-
rate of Ca^{2+}
fluorescent

- (2) Song, H.: 7336-7337
- (3) Zheng, B.: 2523.
- (4) Hatakeyama, T.: 2518
- (5) Cygan, Z.: 3629-3630
- (6) Bringer, S.: 1194.
- (7) Zheng, B.: 11170-11171
- (8) Zheng, B.: 1368.
- (9) Yadin, M.: 900-905.
- (10) Shestopal, S.: 13854-13855
- (11) Chan, E.: 13854-13855
- (12) Hong, L.: *Lan-Chin*
- (13) Song, H.: 7336-7337
- (14) Haeblerli, R.: 13854-13855

Droplet-based microfluidic systems provide a controlled environment in which to perform rapid mixing, isolation of picoliter-size fluid volumes, and rapid variation of reaction conditions. An elegant example of this facility has been demonstrated by Ismagilov and colleagues, in which they used droplets to measure reaction kinetics on the millisecond time scale.¹ The rapid binding

Leng Article



Ismagilov *et al.* did major work concerning droplet microfluidics¹⁹ and concerning the chemistry of the surfactants and oils used to perform the above experiments.⁶¹ Indeed, in order to mimic perfect microbatch conditions, they identified fluorinated oils that were nearly impermeable to water, and synthesized oligoethylene glycol-capped perfluorinated surfactants that prevented protein adsorption at the interface of the droplets. To handle membrane proteins, they also used perfluoroamines that provided high surface tension.

PTX535-006 (reference 61 is Roach)

Roach Article

Anal. Chem. 2005, 77, 785–796

Controlling Nonspecific Protein Adsorption in a Plug-Based Microfluidic System by Controlling Interfacial Chemistry Using Fluorous-Phase Surfactants

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Control of surface chemistry and protein adsorption is important for using microfluidic devices for biochemical analysis and high-throughput screening assays. This paper describes the control of protein adsorption at the liquid–liquid interface in a plug-based microfluidic system. The microfluidic system uses multiphase flows of immiscible fluorous and aqueous fluids to form plugs, which are aqueous droplets that are completely surrounded by fluorocarbon oil but do not come into direct contact with the hydrophobic surface of the microchannel. Protein adsorption at the aqueous–fluorous interface was controlled by using surfactants that were soluble in fluorocarbon oil but insoluble in aqueous solutions. Three perfluorinated alkane surfactants capped with different functional groups were used: a carboxylic acid, an alcohol, and a triethylene glycol group that was synthesized from commercially available materials. Using complementary methods of analysis, adsorption was characterized for several proteins (bovine serum albumin (BSA) and fibrinogen), including enzymes (ribonuclease A (RNAse A) and alkaline phosphatase). These complementary methods involved characterizing adsorption in microfluidic droplets by drop tensiometry and in nanoliter plugs by fluorescence microscopy and kinetic measurements of enzyme catalysis. The oligoethylene glycol-capped surfactant prevented protein adsorption in all cases. Adsorption of proteins to the carboxylic acid-capped surfactant in nanoliter plugs could be described by using the Langmuir model and tensiometry results for microliter drops. The microfluidic system was fabricated using rapid prototyping in poly(dimethylsiloxane) (PDMS). Black PDMS microfluidic devices, fabricated by curing a suspension of charcoal in PDMS, were used to measure the changes in fluorescence intensity more sensitively. This system will be useful for microfluidic bioassays, enzymatic kinetics, and protein crystallization, because it does not require surface modification during fabrication to control surface chemistry and protein adsorption.

This paper describes the biocompatibility of a plug-based microfluidic system obtained through control of surface chemistry

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at the aqueous–fluorous interface. This microfluidic system uses multiphase flows of immiscible fluorous and aqueous liquids to form droplets (plugs) and to transport them with rapid mixing and no Taylor-like dispersion.¹ We have previously used the system to measure enzyme kinetics² and perform protein crystallization^{3–5} using submicroliter volumes of sample.

Microfluidic systems are becoming increasingly useful for a range of analytical^{6–10} and chemical processes,^{11–13} especially systems relying on multiphase flows. Droplet-based microfluidic systems are being developed by a number of groups^{14–20} and

(1) Song, H.; Tice, J. D.; Ismagilov, R. F. *Angew. Chem., Int. Ed.* 2003, 42, 769–772.

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Analytical Chemistry, Vol. 77, No. 3, February 1, 2005 785

10X-000255596

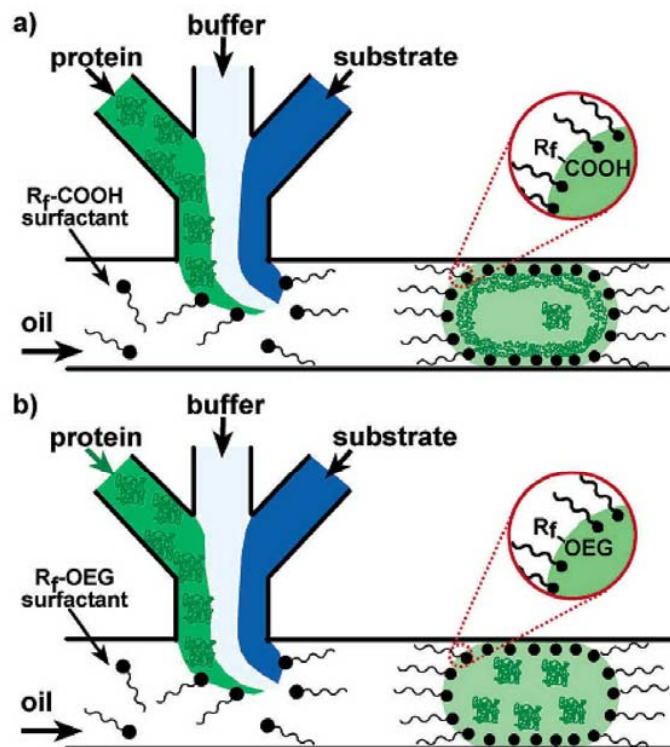


Figure 1. Controlling surface chemistry in plug-based microfluidic devices with surfactants at the aqueous–fluorous interface. (a, b) Schematic of surfactant molecules at the interface of an aqueous plug of protein in a microfluidic channel. (a) R_f -COOH surfactant provided a noninert interface prone to protein adsorption. (b) R_f -OEG surfactant provided an inert, biocompatible interface.

DTX-0286, Fig. 1

Breslauer Article

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Microfluidics-based systems biology

David N. Breslauer, Philip J. Lee and Luke P. Lee

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First published as an Advance Article on the web 9th January 2006
DOI: 10.1039/b515632g

Systems biologists seek to develop a complex understanding of cellular mechanisms by studying the functions of intra- and inter-cellular molecular interactions that trigger and coordinate cellular events. However, the complexity of biological systems makes accurate and precise systems biology experimentation to be a difficult task. Most biological experimentation focuses on highly detailed investigation of a single signaling mechanism, which lacks the throughput necessary to reconstruct the entirety of the biological system, while high-throughput testing often lacks the fidelity and precision necessary to understand the details of a single mechanism. Systems biology experimentation, however, can benefit greatly from the progress in the development of microfluidic devices. Microfluidics provides the opportunity to study cells effectively on both a single- and multi-cell level with high-resolution and localized application of experimental conditions with biometric physiological conditions. Additionally, the ability devices to chip opens the door for high-throughput, high fidelity experimentation and precise unravelling of the intertwined signaling systems that can act on the cell level.

1. Introduction

The post-genomic era has ushered in the promise of complete understanding of biological systems. Systems biology is the discipline currently leading this endeavor, attempting to elucidate the myriad of interactions between the dynamics of molecular components in a biological system such that system behavior can be accurately predicted.¹ To achieve this goal, dynamic models of biological systems must be developed. The approach to model development, as described by Ideker et al.,² is the use of existing data to formulate an initial model and the subsequent use of the model to predict system responses to various perturbations. These perturbations are then experimentally applied to the system and the collected results are used for further model refinement. An example systems model

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David N. Breslauer

David Breslauer is a graduate student in the UCSF/UC Berkeley Bioengineering graduate group. His research interest is primarily in the use of novel micro-scale technologies for the quantitative study of cellular signaling and communication. David earned his BS in Bioengineering from the University of California, San Diego in 2005.

Due to the complexity of biological systems, highly sophisticated methods are required to study them. The development of methodologies and technologies for studying such high-throughput experimental data sets of spatially regulated gene expression techniques and their dynamics by means of chromatin interactions

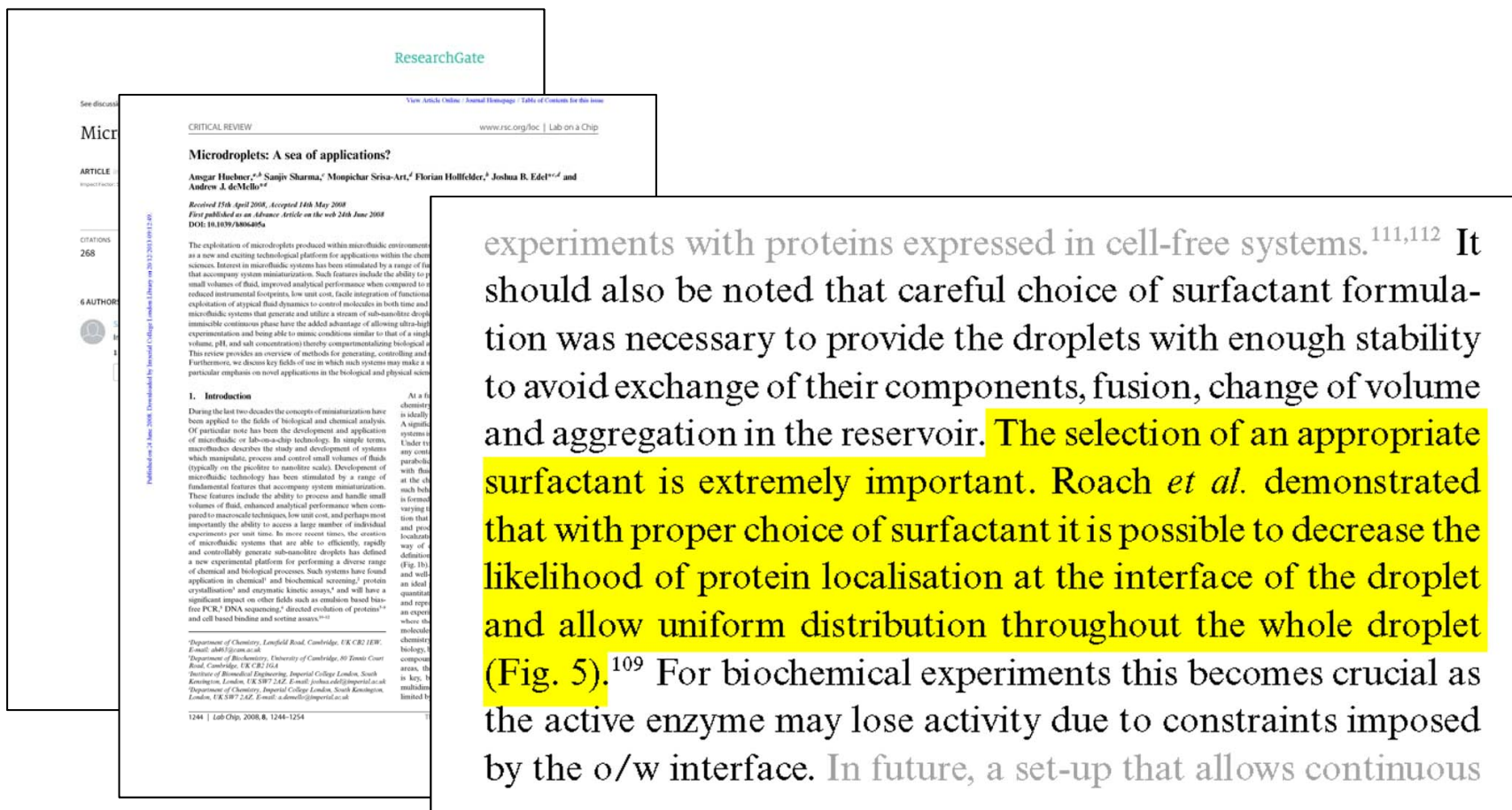
fluoresced, as expected. An important consideration in performing such studies is protein adsorption into the microfluidic device surface, however the same group developed methods of altering droplet interfacial chemistry to actively prevent such problems.⁶³ These developments can be further extended for the creation of artificial membranes on the droplet surface.

OUTSIDE ATTORNEYS' EYES ONLY INFORMATION

RI00113477
PTX536-001

PTX536-010

Heubner Article



PTX989-006 (reference 109 is Roach)

10X's Triblock Surfactant

PTX1202-001

10X's Diblock Surfactant

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

RAINDANCE TECHNOLOGIES, INC.
and THE UNIVERSITY OF CHICAGO,
Plaintiffs and Counterclaim
Defendants,

v.
10X GENOMICS, INC.,
Defendant and Counterclaim
Plaintiff.

C.A. No. 15-152-RG

**OUTSIDE ATTORNEY'S
INFORMATION**

**10X GENOMICS, INC.'S SECOND SUPPLEMENTAL
OBJECTIONS TO RAINDANCE TECHNOLOGIES, INC.'S
CHICAGO'S SECOND SET OF INTERROGATORIES**

Pursuant to Rules 26 and 33 of the Federal Rules of Civil Procedure, Counterclaim Plaintiff 10X Genomics, Inc. ("10X"), based on its understanding, and belief of the facts and on the information available to it, which these Second Supplemental Responses are made, hereby presents responses and objections (collectively, the "Second Supplemental Responses") to RainDance Technologies, Inc.'s (collectively, "Plaintiffs" and each, a "Plaintiff") Chicago's (collectively, the "Interrogatories" and each, an "Interrogatory")

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**PLAINTIFFS'
TRIAL EXHIBIT
PTX1202
C.A. No. 15-152-RG**

PTX1202-001

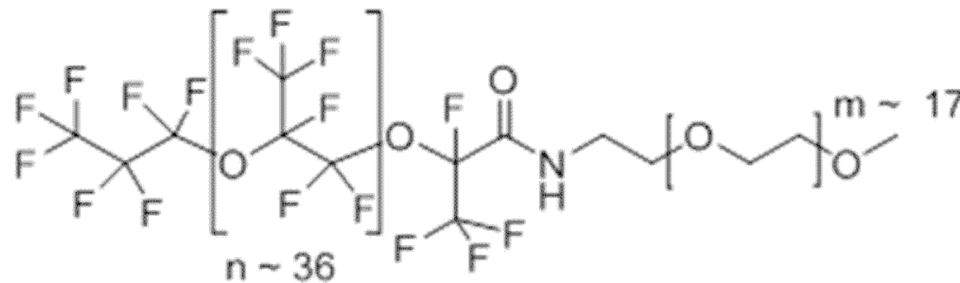


Figure 2. KmPEG

PTX 1202-008 (First Supplemental Response
to Interrogatory No. 7)

Chromium LandLord Reaction

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

RAINANCE TECHNOLOGIES, INC.
and THE UNIVERSITY OF CHICAGO,
Plaintiffs and Counterclaim
Defendants,

v.

10X GENOMICS, INC.,
Defendant and Counterclaim
Plaintiff.

C.A. No. 15-152-RGA

OUTSIDE ATTORNEYS' EYES ONLY
INFORMATION

10X GENOMICS, INC.'S THIRD SUPPLEMENTAL AND CORRECTED FIRST AND
SECOND SUPPLEMENTAL RESPONSES AND OBJECTIONS TO RAINANCE
TECHNOLOGIES, INC. AND THE UNIVERSITY OF CHICAGO'S
INTERROGATORY No. 11

Pursuant to Rules 26 and 33 of the Federal Rules of Civil Procedure, Defendant and
Counterclaim Plaintiff 10X Genomics, Inc. ("10X"), based on its current knowledge,
understanding, and belief of the facts and on the information available to it as of the date on
which these Third Supplemental Responses and Corrected First and Second Supplemental
Responses are made, hereby provides its third supplemental responses and objections
(collectively, the "Third Supplemental Responses" and each, a "Third Supplemental Response"),
corrected first supplemental responses (collectively, the "Corrected First Supplemental
Responses" and each, a "Corrected First Supplemental Response"), and corrected second
supplemental responses (collectively, the "Corrected Second Supplemental Responses," and
each, a "Corrected Second Supplemental Response") to the Second Set of Interrogatories
(collectively, the "Interrogatories" and each, an "Interrogatory") propounded by RainDance

PLAINTIFFS'
TRIAL EXHIBIT
PTX1204
C.A. No. 15-152-RGA

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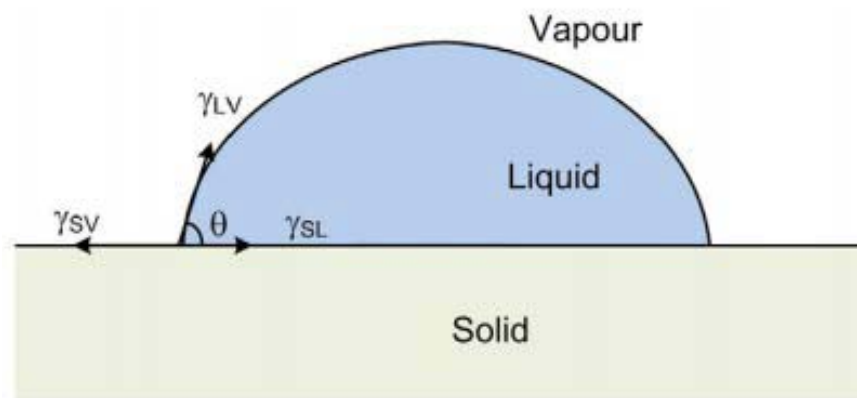
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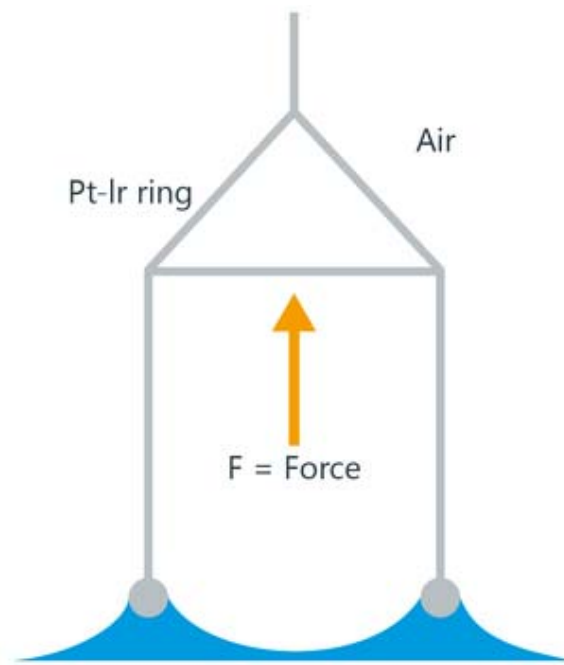
Figure 10.

PTX 1204-011 (Corrected Second Supplemental Response
to Interrogatory No. 11)

Contact Angle Method



Du Noüy Ring Method



Du Noüy Ring Method Apparatus



Solid/Liquid Surface Tensions

	IFT			Average (mN/m)	Maximum	Minimum
1	EG MM	front	DG8	22.2	24.4	20
2		back		19.6	21.6	17.5
3	Probe MM	front	DG8	23.1	25.7	15.8
4		back		24.3	27.8	20.9
5	ddSEQ DNA	front	ddSEQ	17.5	20.8	14.3
6		back		20.4	22	18.8
7	ddSEQ Cell	front	ddSEQ	20.1	23.9	16.4
8		back		18.6	21.2	16
9	Genome Chip V1	front	10X	15.1	18.7	11.5
10		back		21.5	24.4	18.7
11	Genome Chip V2	front	10X	12.5	16	8.9
12		back		26.2	28.2	24.2
13	Gemcode Chip Kit	front	10X	12.4	15.2	9.6
14		back		27.9	31.2	24.6
15	Single Cell V1	front	10X	12.4	14.8	10.1
16		back		19.2	21.2	17.3
17	Single Cell V2	front	10X	18.3	20.5	16.1
18		back		17.6	19.5	15.7

Liquid/Liquid Surface Tensions

	Aqueous sample	Oil sample	Temperature (°C)	IFT (mN/m)
1	Water	HFE-7500	23.93	51.42±0.768
2	Water	DG oil for EG	24.36	5.441 ±0.342
3	Water	DG oil for Probe	24.56	9.048 ±1.317
4	Duplex Assay for Probe	DG oil for Probe	25.28	4.668 ±0.400
5	S. aureus Assay for EvaGreen	DG oil for EG	25.02	2.855 ±0.048
6	ddSEQ with long DNA	DG oil for EG	24.53	2.614 ±0.812
7	ddSEQ with cell sample	DG oil for EG	24.71	3.97 ±1.778
8	Chromium Genome	Chromium Genome Partitioning Oil	25.17	“0”
9	Chromium Genome v2	Chromium Genome v2 Partitioning Oil	25.38	1.81±0.0497*
10	Single Cell A Chip Kit	Single Cell A Chip Kit Partitioning Oil	24.32	“0”
11	Single Cell 3' Chip Kit	Single Cell 3' Chip Kit Partitioning Oil	25.50	“0”
12	GemCode Chip Kit	GemCode Chip Kit Partitioning Oil	25.59	“0”