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Single Cell Sequencing
- Eric Chow (UCSF)

2020 Sept 21-Nature

Webcast: Resolving the

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Single-Cell RNA
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Tutorial: What is Single
Cell RNA-Seq, and why
is it useful? 06 Single
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Understanding chemo
resistance using single
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Capturing Single Cells
with the BD Rhapsody™
Express Single-Cell
Analysis System

Webinar—

~~Characterizing B cells
and their antibodies
using single cell RNA
sequencing (scRNA
seq)~~

Dana Pe'er | Single-Cell
RNA-sequencing | CGSI
2019

Rafael Irizarry,
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Probabilistic Gene
Expression Signatures
for Single Cell RNA seq
DataTALK: Methods
for sample preparation
and single-cell analysis
of solid tumors *Single-
Cell Analysis - Powered
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Analysis Series Part 1*
*CellProfiler - Anne
Carpenter (Broad
Institute)* Flow
cytometry for DNA

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Cytometry Animation 01

~~Single Cell Introduction~~

Single Cell Genomics

Day 2020 - Overview

Next Generation

Sequencing 1: Overview

- Eric Chow (UCSF)

Analysis of single cell

RNA-seq data 23-24

May 2019 *StatQuest: A*

gentle introduction to

RNA-seq Origin of Cells

(IB Bio) (2015)

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*Introduction to Single
Cell RNA-Seq Single
cell analysis: overview,
challenges, solutions
and case studies*

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Atlas - Mapping the
human body one cell at
a time Introduction and
Concepts in Single Cell
Analysis *An overview of
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sequencing and analysis*

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PCR1 Purification

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Sequencing - Finding a

cure for DIPG **Webinar**

Using microfluidic

technologies for DNA

sequencing and single-

cell analysis ~~Single-Cell~~

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through the techniques of standard fluorescence in situ hybridization and polymerase chain reaction (PCR) As the reader moves through the book, the scope and complexity of each technique gradually increases as real-time quantitative PCR ...

~~Single Cell Diagnostics
—Methods and Protocols~~

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~~Alan R...~~

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Single Cell Diagnostics:
Methods and Protocols
applies modern

molecular diagnostic

techniques to the

analysis of single cells,

small numbers of cells,

or cell extracts.

Emphasis is placed on

non-invasive analysis of

single cell metabolites

and the direct analysis

of RNA and DNA from

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tics, with a focus
on polymerase chain
reaction and
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Protocols

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Medical ...~~

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Is Intended For Clinical
And Research Scientists
As Well As Those
Providing Care For
Couples Seeking

Page 17/100

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Treatment For Infertility
Or Preim- Plantation
Genetic Diagnosis. The
Aim Is For All Readers
To Extend Their Knowl-
edge And Expertise In
Analysis Of Single Cells
(whether Or Not That Is
Their ...

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Methods In ...~~

Better resolution and

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Diagnoses are the main advantages of single-cell genome sequencing over microarrays.

Further, sequencing of single cells allows detection of mitochondrial DNA variations. Another study was aimed at observing segmental aneuploidies in trophoctoderm biopsies using a single-cell NGS

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method (Vera-

Rodriguez et al., 2016).

NGS-based methods are also used for

noninvasive prenatal screening to identify aneuploid fetuses before birth.

~~Single-Cell Diagnostics, Prognosis, and Therapy~~

...

Single-cell analysis allows one to isolate

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single nuclei (Evrony et al., 2012) that can then be subjected to amplification followed either by microarray or low-coverage WGS for CNV analysis. The most common method of amplifying DNA from single cells is multiple displacement amplification (MDA) (Dean et al., 2002; Rodrigue et al., 2009).

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A major technical challenge is uneven amplification across the genome, which leads to inaccurate identification of CNVs.

~~Single Cell Analysis—
an overview |~~

~~ScienceDirect Topics~~

Single-cell imagers

These usually scan

single cells or

populations of cells to

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Diagnosic cell
types or, with
Methods And
fluorescent or
Protocols
colorimetric probes, the
presence of specific
proteins or cell features.
They are valuable for
phenotype identification
but are usually very
slow and some can be
quite expensive. Single-
cell sequencing

~~Single Cell Analysis~~

Page 23/100

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~~Advantages, Challenges,~~
and ...

Methods And
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"Single cell diagnostics
has become an

increasingly important
field for both clinicians
and researchers who are
involved in infertility
treatment. ... For
researchers this book
provides not only
detailed state of the art
protocols for single cell
diagnostics, but also

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valuable notes on
troubleshooting and
pitfalls.

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Spatially resolved
proteomic, genomic,
and metabolic profiles
of human cancers are
now possible at the
single-cell level. This
perspective discusses

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spatial bioimaging
methods to decipher the
cascade of
microenvironments in
solid and liquid
biopsies. A unique
synthesis of top-down
and bottom-up analysis
methods is presented.

~~Multiplex bioimaging of
single-cell spatial
profiles for ...~~

As the technologies for

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Diagnosing bio-molecular components in single cells are being

developed, single cell analysis seems

promising to address the current limitations due to averaging problems.

Although the technologies for single cell analysis are still at the infant stage, the single cell approach has the potential to improve

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the accuracy of
diagnosis based on
knowledge of intra- and
inter-cellular networks.

~~Emerging applications
of single-cell
diagnostics.~~

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Methods in Molecular
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Methods and Protocols

Methods in ...

Many single-cell analysis techniques require the isolation of individual cells.

Methods currently used for single cell isolation include:

Dielectrophoretic digital sorting, enzymatic

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digestion, FACS,
hydrodynamic traps,
laser capture
microdissection, manual
picking, microfluidics,
micromanipulation,
serial dilution, and
Raman tweezers.

~~Single cell analysis~~

~~Wikipedia~~

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Here, we benchmarked
22 classification
methods that
automatically assign cell
identities including
single-cell-specific and
general-purpose
classifiers. The

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performance of the methods is evaluated using 27 publicly available single-cell RNA sequencing datasets of different sizes, technologies, species, and levels of complexity.

~~A comparison of automatic cell identification methods for ...~~

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This book applies modern molecular diagnostic techniques to the analysis of single cells, small numbers of cells, or cell extracts. Emphasis is placed on non-invasive analysis of single cell metabolites and the direct analysis of RNA and DNA from single cells, with a focus on polymerase chain reaction and

Read Online Single Cell fluorescence in situ hybridization. Methods And Protocols

This book applies modern molecular diagnostic techniques to the analysis of single cells, small numbers of cells, or cell extracts. Emphasis is placed on non-invasive analysis of single cell metabolites

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Diagnostic and the direct analysis of RNA and DNA from single cells, with a focus on polymerase chain reaction and fluorescence in situ hybridization. In particular, this handbook is essential for practitioners providing care for couples seeking treatment for infertility.

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Single-cell omics is a progressing frontier that stems from the sequencing of the human genome and the development of omics technologies, particularly genomics, transcriptomics, epigenomics and proteomics, but the sensitivity is now improved to single-cell level. The new

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Diagnosics

Methodologies,

especially the next

generation sequencing

(NGS) technology,

plays a leading role in

genomics related fields;

however, the

conventional techniques

of omics require number

of cells to be large,

usually on the order of

millions of cells, which

is hardly accessible in

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Diagnos some cases. More importantly, harnessing the power of omics technologies and applying those at the single-cell level are crucial since every cell is specific and unique, and almost every cell population in every systems, derived in either vivo or in vitro, is heterogeneous.

Deciphering the

Page 42/100

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Methods And
Protocols

heterogeneity of the cell population hence becomes critical for recognizing the mechanism and significance of the system. However, without an extensive examination of individual cells, a massive analysis of cell population would only give an average output of the cells, but neglect

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the differences among cells. Single-cell omics seeks to study a number of individual cells in parallel for their different dimensions of molecular profile on genome-wide scale, providing unprecedented resolution for the interpretation of both the structure and function of an organ,

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tissue or other system,
as well as the interaction
(and communication)
and dynamics of single
cells or subpopulations
of cells and their
lineages. Importantly
single-cell omics
enables the
identification of a minor
subpopulation of cells
that may play a critical
role in biological
process over a dominant

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Diagnostic such as a cancer and a developing organ. It provides an ultra-sensitive tool for us to clarify specific molecular mechanisms and pathways and reveal the nature of cell heterogeneity. Besides, it also empowers the clinical investigation of patients when facing a very low quantity of cell available for analysis,

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such as noninvasive cancer screening with circulating tumor cells (CTC), noninvasive prenatal diagnostics (NIPD) and preimplantation genetic test (PGT) for in vitro fertilization. Single-cell omics greatly promotes the understanding of life at a more fundamental level, bring vast applications in

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medicine. Accordingly, single-cell omics is also called as single-cell analysis or single-cell biology. Within only a couple of years, single-cell omics, especially transcriptomic sequencing (scRNA-seq), whole genome and exome sequencing (scWGS, scWES), has become robust and broadly accessible.

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Besides the existing technologies, recently, multiplexing barcode design and combinatorial indexing technology, in combination with microfluidic platform exemplified by Drop-seq, or even being independent of microfluidic platform but using a regular PCR-plate, enable us a greater

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capacity of single cell analysis, switching from one single cell to thousands of single cells in a single test. The unique molecular identifiers (UMIs) allow the amplification bias among the original molecules to be corrected faithfully, resulting in a reliable quantitative measurement of omics

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in single cells. Of late, a variety of single-cell epigenomics analyses are becoming sophisticated, particularly single cell chromatin accessibility (scATAC-seq) and CpG methylation profiling (scBS-seq, scRRBS-seq). High resolution single molecular Fluorescence in situ hybridization (smFISH)

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and its revolutionary versions (ex. seqFISH, MERFISH, and so on), in addition to the spatial transcriptome sequencing, make the native relationship of the individual cells of a tissue to be in 3D or 4D format visually and quantitatively clarified. On the other hand, CRISPR/cas9 editing-based In vivo lineage

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Diagnostic methods enable dynamic profile of a whole developmental process to be accurately displayed. Multi-omics analysis facilitates the study of multi-dimensional regulation and relationship of different elements of the central dogma in a single cell, as well as permitting a clear dissection of the

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Diagnosics

heterogeneity of a system. Last but not the least, the technology,

biological noise, sequence dropout, and batch effect bring a huge challenge to the bioinformatics of single cell omics. While

significant progress in the data analysis has been made since then, revolutionary theory and

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algorithm logics for single cell omics are expected. Indeed, single-cell analysis exert considerable impacts on the fields of biological studies, particularly cancers, neuron and neural system, stem cells, embryo development and immune system; other than that, it also tremendously motivates

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pharmaceutical RD,
clinical diagnosis and
monitoring, as well as
precision medicine. This
book hereby
summarizes the recent
developments and
general considerations
of single-cell analysis,
with a detailed
presentation on selected
technologies and
applications. Starting
with the experimental

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Design on single-cell omics, the book then emphasizes the consideration on heterogeneity of cancer and other systems. It also gives an introduction of the basic methods and key facts for bioinformatics analysis. Secondary, this book provides a summary of two types of popular technologies,

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the fundamental tools on single-cell isolation, and the developments of single cell multi-omics, followed by descriptions of FISH technologies, though other popular technologies are not covered here due to the fact that they are intensively described here and there recently. Finally, the book illustrates an elastomer-

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based integrated fluidic circuit that allows a connection between single cell functional studies combining stimulation, response, imaging and measurement, and corresponding single cell sequencing. This is a model system for single cell functional genomics. In addition, it reports a pipeline for

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Diagnostic proteomics with an analysis of the early development of *Xenopus* embryo, a single-cell qRT-PCR application that defined the subpopulations related to cell cycling, and a new method for synergistic assembly of single cell genome with sequencing of amplification product by phi29 DNA polymerase.

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Due to the tremendous progresses of single-cell omics in recent years, the topics covered here are incomplete, but each individual topic is excellently addressed, significantly interesting and beneficial to scientists working in or affiliated with this field.

Single-cell Omics,
Volume 2: Advances in
Page 61/100

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Diagnosics provides the latest single-cell omics applications in the field of biomedicine.

The advent of omics technologies have enabled us to identify the differences between cell types and subpopulations at the level of the genome, proteome, transcriptome, epigenome, and in

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several other fields of omics. The book is divided into two sections: the first is dedicated to biomedical applications, such as cell diagnostics, non-invasive prenatal testing (NIPT), circulating tumor cells, breast cancer, gliomas, nervous systems and autoimmune disorders, and more. The second

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focuses on cell omics in plants, discussing micro algal and single cell omics, and more. This book is a valuable source for bioinformaticians, molecular diagnostic researchers, clinicians and several members of biomedical field interested in understanding more about single-cell omics

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and its potential for
research and diagnosis.

Covers the diverse
single cell omics

applications in the
biomedical field

Summarizes the latest
progress in single cell
omics and discusses

potential future
developments for
research and diagnosis

Written by experts
across the world, it

Page 65/100

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brings different points-of-view and study cases to fully give a comprehensive overview of the topic

This book summarizes the various microfluidic-based approaches for single-cell capture, isolation, manipulation, culture and observation, lysis, and analysis.

Single-cell analysis

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Diagnosics

reveals the heterogeneities in morphology, functions, composition, and

genetic performance of seemingly identical

cells, and advances in single-cell analysis can

overcome the

difficulties arising due to cell heterogeneity in

the diagnostics for a

targeted model of

disease. This book

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provides a detailed review of the state-of-the-art techniques presenting the pros and cons of each of these methods. It also offers lessons learned and tips from front-line investigators to help researchers overcome bottlenecks in their own studies. Highlighting a number of techniques, such as microfluidic

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Droplet techniques,
combined microfluidics-
mass-spectrometry
systems, and

nanochannel sampling,
it describes in detail a
new microfluidic chip-
based live single-cell
extractor (LSCE)

developed in the
editor's laboratory,
which opens up new
avenues to use open
microfluidics in single-

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Protocols
cell extraction, single-cell mass spectrometric analysis, single-cell adhesion analysis and subcellular operations.

Serving as both an elementary introduction and advanced guidebook, this book interests and inspires scholars and students who are currently studying or wish to study microfluidics-

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based cell analysis
methods.

Biosensors for Single-Cell Analysis explores a wide range of biosensor technologies and their applications in single-cell characterization and analysis. Sections cover key biophysical and chemical single-cell

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properties that consider proteomic, metabolic, electrical, mechanical and optical properties.

Each chapter features key definitions and case studies, providing detailed guidance for researchers who want to replicate covered solutions in their work.

Tutorial sections, evaluations of the current state-of-the-field

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Diagnos...
and future developments
are also included.

Methods And
Protocols
Microfluidic approaches
to characterization, such

as microfluidic

impedance flow

cytometry and

microfluidic flow

cytometry are

considered alongside

more conventional

approaches, such as

mass spectroscopy,

fluorescent and mass

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flow cytometry.

Additionally, key types of biosensors are covered, including

atomic force

microscopy,

micropipette aspiration,

optical tweezers,

microfluidic

hydrodynamic

stretchers, microfluidic

constriction channel and

microfluidic optical

stretchers. Includes

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chapters focused on key
single-cell properties,
such as proteomic,
metabolic and
mechanical
characterization

Features case studies
that illustrate the
application of
biosensors for single-
cell analysis Considers
microfluidic approaches
for each single-cell
property discussed

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Explores future
directions for single-cell
analysis and biosensor
technology
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Single-Cell Omics:

Volume 1:

Technological Advances
and Applications

provides the latest
technological

developments and
applications of single-
cell technologies in the

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field of biomedicine. In the current era of precision medicine, the single-cell omics technology is highly promising due to its potential in diagnosis, prognosis and therapeutics. Sections in the book cover single-cell omics research and applications, diverse technologies applied in the topic, such as

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Pangenomics,

metabolomics, and multi-omics of single cells, data analysis, and

several applications of single-cell omics within the biomedical field, for example in cancer, metabolic and neuro diseases, immunology, pharmacogenomics, personalized medicine and reproductive health.

This book is a valuable

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source for
bioinformaticians,
molecular diagnostic
researchers, clinicians
and members of the
biomedical field who
are interested in
understanding more
about single-cell omics
and its potential for
research and diagnosis.
Covers not only the
technological aspects,
but also the diverse

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Applications of single
cell omics in the
biomedical field

Summarizes the latest
progress in single cell
omics and discusses
potential future
developments for
research and diagnosis

Written by experts
across the world,
bringing different points-
of-view and case studies
to give a comprehensive

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overview on the topic

Methods And

Protocols
One of the key
challenges of biology is

to understand how
individual cells process
information and respond
to perturbations.

However, most of the
existing single cell
analysis methods can
only provide a glimpse
of cell properties at
specific time points and

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are unable to provide cell secretion and protein analysis at the single cell resolution.

This thesis offers the description of a single-cell assay as well as a CO₂-induced enrichment method for the analysis of single cells secretions. The single-cell assay introduced in this thesis enables the

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accommodation of
different cellular types,
allows for easy and
efficient single cell
loading and culturing,
and is suitable for
studying the effects of in-
vitro environmental
factors in combination
with drug screening.
One salient feature of
the assay is the non-
invasive collection and
survey of single cell

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secretions at different time points, producing unprecedented insight of single cell behaviors based on the biomarker signals from individual cells under given perturbations. In addition, the open-well design of the assay allows for simple collection of cells with standard tools such as pico-pipette for

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downstream processes in relating the single-cell secretions with gene analysis. Above all, the acquired information is quantitative. For example, measured by the number of exosomes each single cell secretes for a given time period, exosomal miRNA carried by exosomes secreted by single cell. Therefore, this single-

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cell assay provides a convenient, low-cost, and robust tool for quantitative, time lapsed studies of single cell properties. Another challenge for single cell secretion analysis is the limit-of-detection (LOD) and sensitivity. Thus, sample enrichment is an important step in the work flow of biosensing

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Diagnosis for disease detection and numerous biological or clinical processes. Most current techniques require devices that are tailored to specific chemical or physical characteristics of the target objects to enrich or capture them from the sample. The complexity within these devices all serve to, increase cost and may

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Diagnos

even limit the enrichment factor. Here, a technique of using a CO₂ laser to drive

targets towards the laser

spot via mass transport

without requiring any

device fabrication

processes or special

reagents was introduced.

To prove the concept,

single-stranded DNA

(ssDNA) has been

enriched by more than

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100,000-fold in less than 4 minutes. The temperature and evaporation rate profile at the enriched area are measured alongside theoretical analyses and modeling to monitor and understand the physical process. The formation of aggregates comprised of streptavidin Q-dots and biotin labeled exosomes with this

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method was demonstrated to show the capability of

biosample detection,

purification, and

quantification. The

method is not only

simple and highly

efficient, but also

applicable to all types of

biomolecules and

bioparticles. Thereby

promising a simple, cost

effective and efficient

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solution for biological
sample preparation for
sensing, analytics, and
diagnostics.

This book summarizes
the various microfluidic-
based approaches for
single-cell capture,
isolation, manipulation,
culture and observation,
lysis, and analysis.
Single-cell analysis
reveals the

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heterogeneities in morphology, functions, composition, and genetic performance of seemingly identical cells, and advances in single-cell analysis can overcome the difficulties arising due to cell heterogeneity in the diagnostics for a targeted model of disease. This book provides a detailed

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review of the state-of-the-art techniques presenting the pros and cons of each of these methods. It also offers lessons learned and tips from front-line investigators to help researchers overcome bottlenecks in their own studies. Highlighting a number of techniques, such as microfluidic droplet techniques,

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tic microfluidics-
mass-spectrometry
systems, and

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nanochannel sampling,

it describes in detail a

new microfluidic chip-

based live single-cell

extractor (LSCE)

developed in the editor's

laboratory, which opens

up new avenues to use

open microfluidics in

single-cell extraction,

single-cell mass

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spectrometric analysis,
single-cell adhesion
analysis and subcellular
operations. Serving as
both an elementary
introduction and
advanced guidebook,
this book interests and
inspires scholars and
students who are
currently studying or
wish to study
microfluidics-based cell
analysis methods.

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Diagnostics

PEM Fuel Cell Testing
and Diagnosis covers

the recent advances in

PEM (proton exchange
membrane) fuel cell

systems, focusing on
instruments and

techniques for testing

and diagnosis, and the

application of diagnostic

techniques in practical

tests and operation. This

book is a unique source

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of electrochemical techniques for researchers, scientists and engineers working in the area of fuel cells.

Proton exchange membrane fuel cells are currently considered the most promising clean energy-converting devices for stationary, transportation, and micro-power applications due to their

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high energy density, high efficiency, and environmental friendliness. To advance research and development of this emerging technology, testing and diagnosis are an essential combined step. This book aids those efforts, addressing effects of humidity, temperature and pressure on fuel cells,

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Diagnosis and failure analysis, and design and assembly of MEAs, single cells and stacks.

Provides fundamental and theoretical principles for PEM fuel cell testing and diagnosis.

Comprehensive source for selecting techniques, experimental designs and data analysis

Analyzes PEM fuel cell

Read Online

Single Cell

Degradation and failure mechanisms, and suggests failure mitigation strategies

Provides principles for selecting PEM fuel cell key materials to improve durability

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