

nadia

an innovation in
high throughput
single cell profiling



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Why use high throughput single cell profiling?

Techniques such as high throughput scRNA-Seq (single cell RNA sequencing) offer an unprecedented combination of quality and quantity of single cell data

For example, heterogeneous samples from tissue, blood etc. containing thousands of cells can be rapidly profiled at the single cell level. This enables the quantification of gene expression and in turn the identification and quantification of individual cell types.

High throughput profiling of paired V(D)J transcripts from B cells and T cells enables the investigation of diversity and clonality in an antibody or TCR repertoire. This offers useful insights into immune responses and reactions.



Why choose Nadia?

Nadia, developed by Dolomite Bio, takes high throughput scRNA-Seq to the next level by using innovation to solve a vast range of industry-wide challenges – see Nadia Technology Highlights on page 8.

By automatically encapsulating single cells in microfluidic droplets with mRNA capture beads, Nadia generates up to 48,000 barcoded single cell mRNA libraries in 15 minutes. After sequencing, this enables detailed profiling of large heterogeneous cell populations.

Adding the Nadia Innovate upgrade to the Nadia Instrument transforms it into a system for the development of novel protocols and applications.

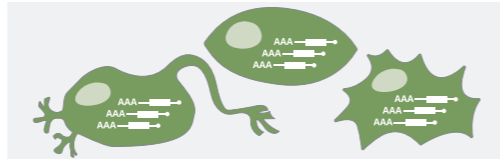
Dolomite Bio's in-house team of biologists and worldwide network of local specialists work with you to provide advice for your application, product demonstrations, installation, training and support.

scRNA-Seq Workflow

1. Upstream

SAMPLE PREPARATION

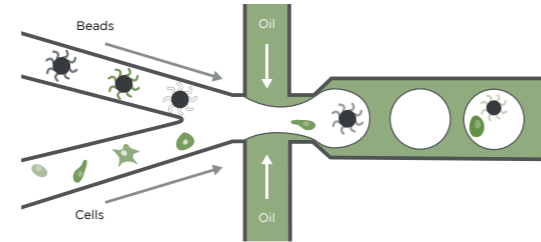
Prepare suspension of single cells or nuclei from virtually any eukaryotic sample e.g. tissues, blood, biopsies, tumours, cultured cells, plants, yeasts, protoplasts, etc.



2. Nadia Instrument

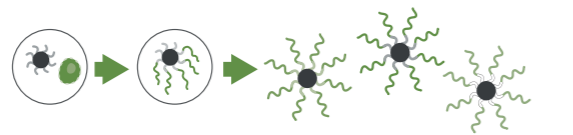
MICROFLUIDIC COMPARTMENTALIZATION OF CELLS WITH BARCODED mRNA CAPTURE BEADS

Thousands of single cells are individually compartmentalized with barcoded oligo beads in droplets. To achieve this, separate suspensions of cells and beads are combined before being dropletized in oil on a microfluidic chip.

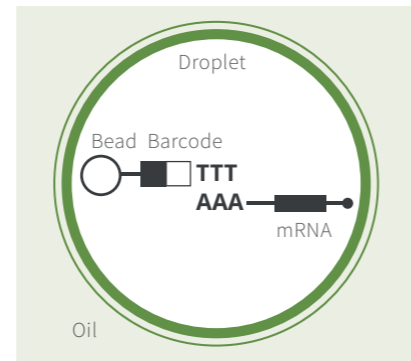
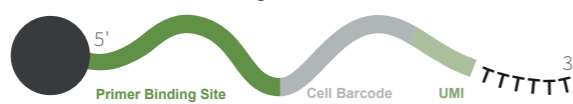


CELL LYSIS AND mRNA CAPTURE

Inside a given droplet, an individual cell is lysed and its mRNA is captured on a single uniquely barcoded bead. The droplets are collected in the on-chip reservoir.



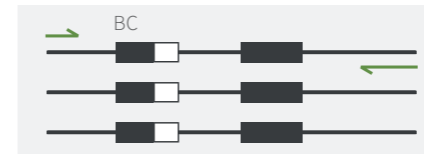
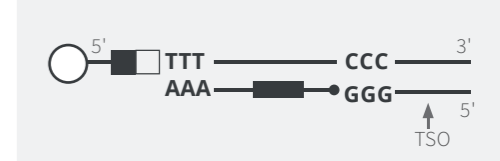
Structure of the Barcoded Oligonucleotide



3. Downstream

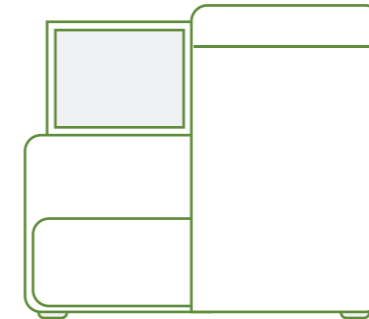
FIRST STRAND cDNA SYNTHESIS

Off chip, the droplets are broken and the single cell mRNA barcoded beads are recovered in bulk. The barcoded oligo on the bead primes reverse transcription of the mRNA. The resulting bead-bound single cell cDNA libraries are uniquely barcoded by cell-of-origin.



LIBRARY AMPLIFICATION

The barcoded cDNAs are amplified in bulk with primers annealing to the primer binding sites on the bead oligo sequence and the Template Switch Oligo (TSO) sequence. This results in a pool of the thousands of single cell cDNA libraries.



Cell:	1	2	...	N
GENE 1	1	2		14
GENE 2	4	27		8
GENE 3	0	0		1
...
GENE M	6	2		0

NGS SEQUENCING

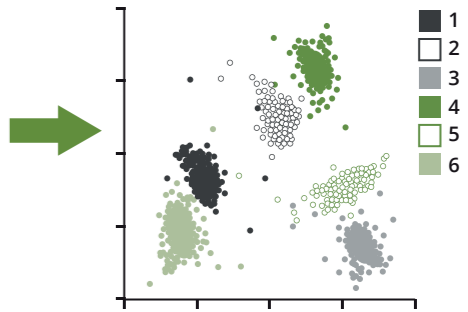
The pooled barcoded cDNA libraries are typically processed with a Nextera® kit and sequenced using an Illumina® sequencer i.e. HiSeq®4000/2500/NextSeq®/MiSeq®. The libraries may be also be processed for sequencing on other platforms e.g. for full length sequencing for analysis of alternate splicing.

BIOINFORMATICS PIPELINE

A digital gene expression matrix is generated using an established pipeline: 1) Sequences are assigned to genes by aligning to the genome and the results grouped by barcode (cell). 2) Unique Molecular Identifiers are counted for each gene in each cell to determine transcript abundance.

DATA VISUALIZATION

A typical approach includes t-stochastic neighbour embedding (t-SNE), a nonlinear dimensionality reduction technique to enable 2D (or 3D) visualization of single cell clusters.



The Nadia Instrument

The Nadia Instrument is a microfluidic droplet based platform for single cell research that automatically runs 1, 2, 4 or 8 cell samples in parallel in ~15 mins. Each sample generates up to 6,000 high quality single cell libraries.

After automatically detecting an application-specific microfluidic cartridge e.g. for Drop-seq, the Nadia Instrument guides users through sample loading via the touchscreen user interface and runs the samples automatically.



Benefits:

- **Flexibly Configurable:** The Nadia Instrument can run standard protocols, or be transformed into a configurable system, when used with Nadia Innovate
- **Easy to use:** Automatic detection of application-specific cartridges of chips, touch screen interface and sample loading guide lights under the chip
- **Truly single cell:** Ultra low cell doublet rates due to gentle cell agitation
- **Variable sample size:** Run 1, 2, 4 or 8 samples in parallel
- **High throughput:** Run up to 8 samples in parallel with automation in ~15 mins
- **High quality results:** Automated sample chilling maintains transcriptome state
- **No cross contamination:** Uses disposable microfluidic chips and has no wetted instrument parts
- **Wide range of applications:** Instrument is designed for a wide variety of applications, automatically controlling flow rate, temperature, agitation and timings dependent upon the application
- **Automated:** Fully automated sample encapsulation steps
- **Elegant user interface:** Guides the user through sample loading steps

Nadia Technology Highlights

The Nadia product family elegantly solves the range of industry-wide single cell profiling challenges by implementing a range of technological innovations.

OIL U-BEND

The oil well U-bend is proprietary technology that avoids unwanted oil wicking into the microfluidic channel before the run starts. This ensures the highest quality droplets and minimizes cell doublets. Also, by a novel automated oil priming step, wicking of aqueous fluids is also avoided.

SAMPLE LOADING PORTS

Easy sample loading is achieved by ports designed to accept standard pipette tips.

BARCODED mRNA CAPTURE BEADS

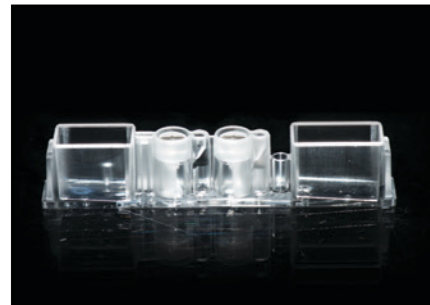
Beads are needed with unique bead barcodes and UMIs (Unique Molecular Identifiers) to ensure barcoding of cDNA and elimination of the effects of amplification bias.

HIGH THROUGHPUT DROPLET JUNCTION

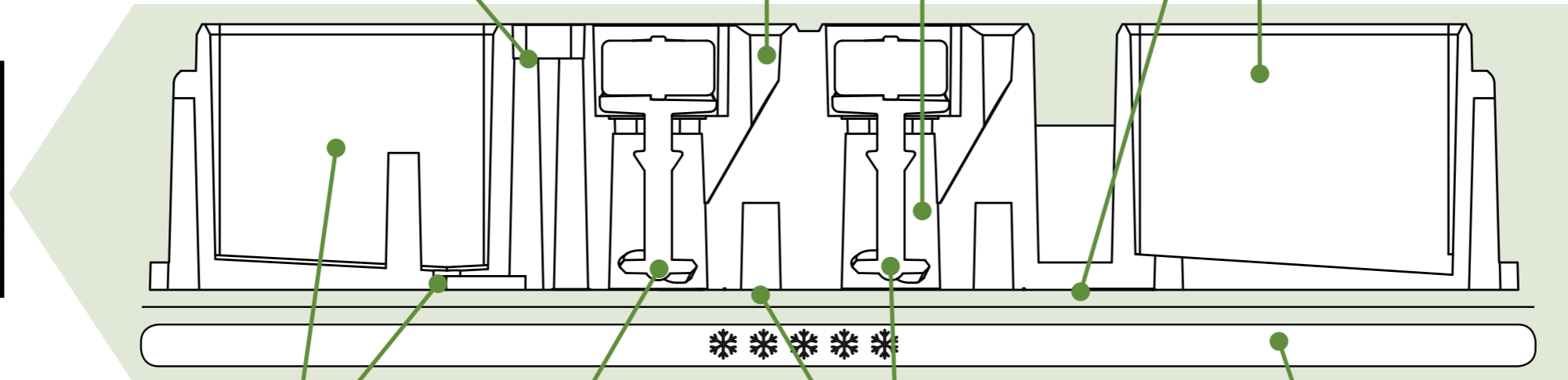
Approximately 6000 cells are co-encapsulated with a bead per channel. Live junction visualisation is enabled when using the Nadia Innovate.

MULTIPLE CHIPS

1, 2, 4 or 8 identical chips can be held in one cartridge. Multiple separate chips ensure greater run-to-run consistency by ensuring the microfluidic pathways are identical.



Above: Nadia cartridge chip
Right: Cross-section of Nadia cartridge chip



PRESSURE PUMPS

The ultra-smooth pressure pumps ensure the most monodisperse droplets possible, thereby maximizing the number of single cells captured. This is achieved by avoiding doublets in larger droplets and avoiding cells not encapsulated with a bead in smaller droplets.

OIL AND SURFACTANT

Biocompatible oil and emulsion stabiliser are needed to enable droplet formation.

FILTERS

On-chip filters allow cells and beads through but filter out fibres and other particulates, maximizing successful runs.

CELL STIRRER

In built stirrers gently agitate the cell samples during the run. This proprietary technology maximizes data quality (by minimizing cell doublets and ensuring an even distribution of single cells throughout the run) and maximizes successful runs by avoiding blockages. The stirrers avoid cell damage by rotating gently and away from walls or bottom of the well.

BEAD STIRRERS

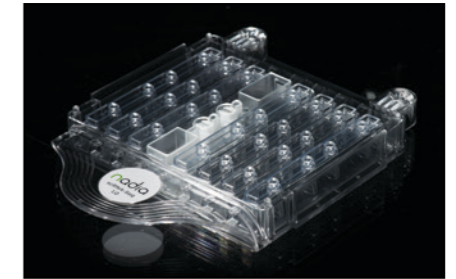
Beads are stirred to ensure singulation and an even distribution of beads throughout the run.

GUIDE LIGHTS

Under-chip guide lights and a wizard-style touchscreen user interface indicate which wells to fill at each step, avoiding potential user errors.

TEMPERATURE CONTROLLER

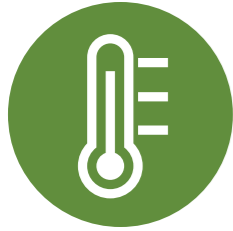
The inbuilt temperature controller maintains the cell transcription profile by chilling samples throughout the run. It also minimizes cell doublets by eliminating the effect of ambient temperature changes and hence changes in droplet size.



Nadia Instrument Features:



3 independent ultra-smooth pressure pumps each up to 1 bar



Chip temperature control from 4°C to 40°C



Independent gentle stirring of beads and cells prior to encapsulation



Easy to use integrated touch screen interface



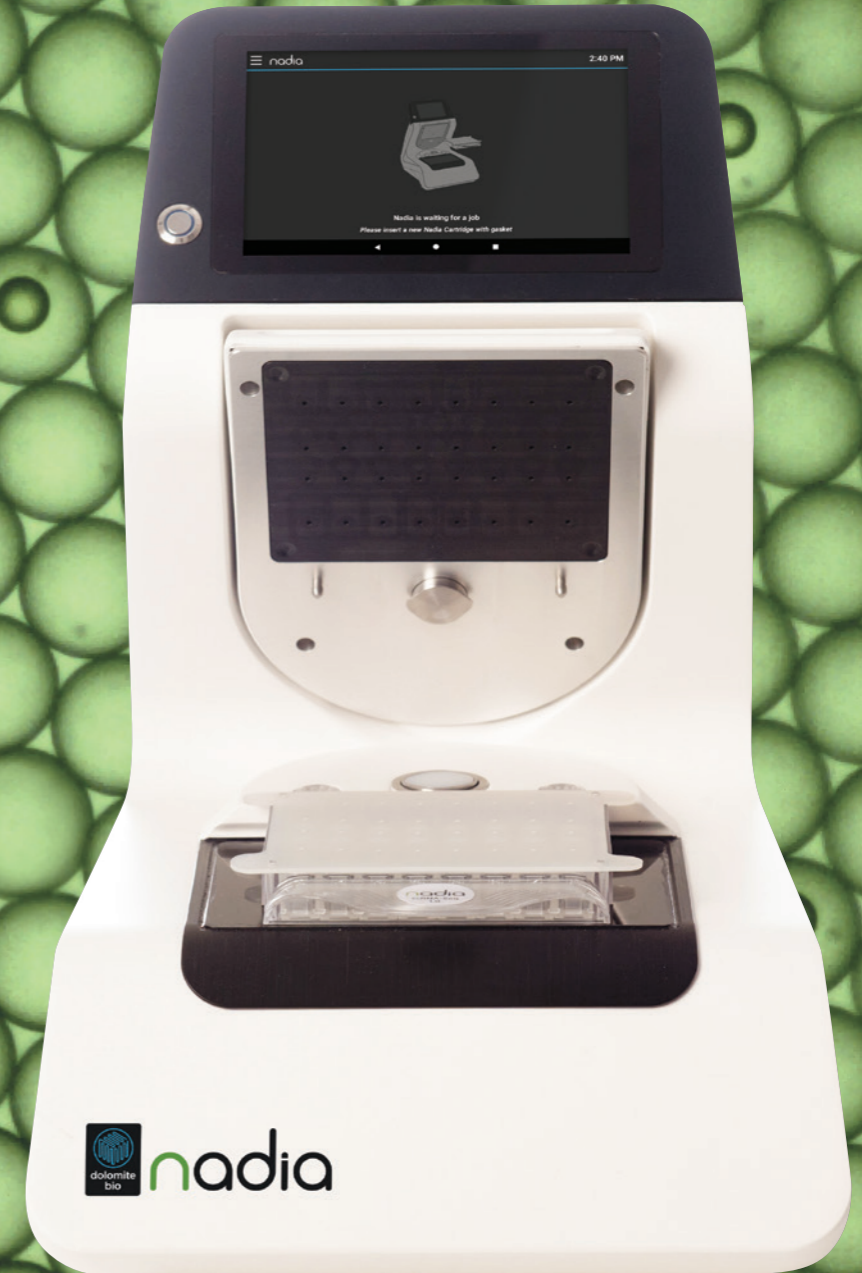
Step-by-step tutorial software



Disposable cartridges prevent cross contamination



Automatic detection of application-specific cartridges of microfluidic chips



Nadia Innovate

Nadia Innovate is a protocol development module which is easily connected to the Nadia Instrument, allowing the user to develop new single cell protocols and applications. Once validated using Nadia Innovate, protocols can be transferred to the Nadia Instrument for high throughput parallel operation. By allowing user control of parameters (such as droplet size, droplet frequency, temperature, agitation and timings), innovation is unlocked.



Benefits:

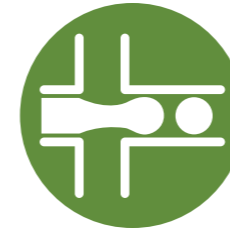
- **Open development system:** Turns the Nadia Instrument into a system for development of new protocols and applications
- **Scalable:** Enables scalability from a single chip to 2, 4 or 8 chips in parallel when transferring protocol to the Nadia Instrument
- **Quick and easy set-up of experiments**
- **Rapid protocol optimization:** Quickly vary droplet size, frequency, droplet components, temperature, times and agitation
- **Easy visualization of process:** Use the high-speed microscope and camera to see real-time droplet formation
- **Flexible PC software:** After automating one experiment at a time, the software can apply the same conditions to many experiments in parallel



Nadia Innovate Features:



Integrated temperature control from 4 – 40°C



Ability to visualise droplet formation at the junction



Option for viewing of whole chip from underside when used with an inverted microscope



Integrated stirring of 2 aqueous reservoirs



P-pump control of 3 independent channels up to 1 bar



High speed camera allows imaging and capture of droplet formation



Use single chips on the Nadia Innovate Module to optimize protocol, then run 2, 4 or 8 samples in parallel on the Nadia Instrument



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