Versatility at your fingertips

Unprecedented flexibility for rapid protocol optimization & application development

Get started guide

nadia

dolomite bio

Customizing single cell research

Tailor your research to an unlimited range of projects, applications and cell types

The recent development of high-throughput single cell techniques has allowed the analysis of thousands of individual cells in parallel. With this unprecedented approach, researchers are now able to identify cellular heterogeneity and analyse gene expression patterns across a variety of samples and application areas in order to characterize and identify cell types, cell states and cell-to-cell interactions. Whilst most commercially available instruments and protocols focus on commonly used cell types with predefined consumables and protocols, cells obtained from plants or other non-model organisms may require protocol adjustments of the single cell capture step due to cell size, buffer compositions or other variations. In addition, scientists are now looking to increasingly customize existing protocols or develop new protocols and applications to fit with their specific experimental needs. In order to take full advantage of high-throughput single cell research capabilities, versatile and flexible technology is required.

With Dolomite Bio's Nadia Innovate, researchers are empowered to customize and tailor protocols and reagents to their research needs, thus enabling rapid development of new applications and protocols for a wide range of cell types.

This guide is designed to help customers fine tune their single cell research based on their sample and research requirements using the Nadia Innovate. It provides a roadmap to optimize and change a range of different parameters such as droplet size, flow rates and temperature to enable the design of customized protocols and development of new applications.

Easy software-controlled adjustments of critical parameters such as temperature, stirrer speed, flow pressures and incubation times during droplet formation

Temperature

Temperature control is essential in many biological applications, and requirements vary depending on experimental needs. For example, whilst keeping a cell sample at cold temperature helps maintain the transcriptome state, the production of hydrogel beads with agarose requires heating to keep the agarose molten.

Stirring speed

To ensure an even distribution of cells and beads (if using beads) in droplets throughout a run, it is crucial to keep both in suspension as they will otherwise settle at the bottom of their respective wells, resulting in higher doublet rates. On the Nadia Innovate, stirring of cells and beads can be controlled individually, and stirring speeds can be changed dependent on cell and/or bead densities and properties.

Pressure

A significant benefit of the Nadia Innovate platform is that users have the flexibility to develop and optimize protocols based on their chosen cells and experimental requirements, including adjustments of droplet sizes based on cell size, or pressure table adjustments based on buffer compositions. Specifically, when studying non-model, i.e. non-mammalian, organisms, the use of standard buffers such as PBS is often not recommended. Plant protoplasts, for example, require a viscous buffer with high osmolyte contents to prevent early cell lysis. Modifications in the composition of the droplet buffers will cause these reagents to behave differently in the microfluidic channel and so require the user to adjust pressures to achieve stable droplet formation.

Time

Along with temperature control, timing is important in biology applications. Pre- and post-run incubation steps can be created to suit the user's specific experiment, e.g. the addition of a post-run incubation step for improved cell lysis. Furthermore, the total duration of a run can be increased or decreased to process more, or less, sample, and accommodate for various sample sizes, ranging from 100 to 250 µl.

Temperature	Aqueous stirring speed (for cells and beads)	Pressure	Time (run/ incubation/pre-run)
4-40 degrees	0-300 rpm	0-1000 mbar	User defined

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Pressure adjustments to adapt to reagent viscosity

The Nadia Innovate enables the adjustment of pressures. This is beneficial when using a buffer with a different viscosity compared to the reference buffer; or when using deformable beads as these require different pressures to achieve optimal flow rates.

- When using a buffer with **higher** viscosity compared to Dolomite Bio's scRNA-Seq on Nadia cell buffer, the flow rate will decrease and the interface between the two aqueous phases will move away from the middle of the aqueous channels. To equilibrate the two streams, slowly **increase the pressure** in the relevant channel until the interface is back in the middle.
- When using a buffer with **lower** viscosity compared to Dolomite Bio's scRNA-Seq on Nadia cell buffer, the flow rate will increase and the interface between the two aqueous streams will move away from the middle of the aqueous channels. To equilibrate the two phases, slowly **decrease the pressure** in the relevant channel until the interface is back in the middle.
- When using deformable beads, slowly **increase the pressure** in the beads channel until the beads reach the junction.

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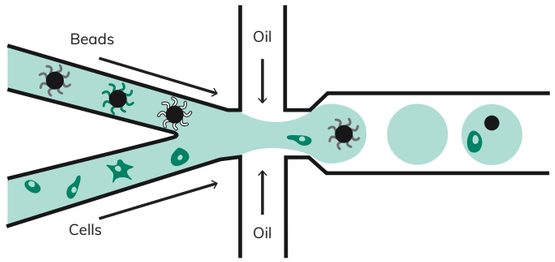
Change of detergent type or concentration

Different detergent types or concentrations can be used with the Nadia Innovate. Some adjustments will be needed in pressure to optimize experiments and prevent jetting. Jetting is observed when the aqueous phases form a long streak of droplets after the junction or, in some extreme cases, not at all.

- When increasing the concentration of a detergent, jetting is more likely to happen. To stop this, **decrease the pressure** in the relevant channel.
- If jetting is observed when changing the type of detergent used, **decrease the pressure** in the relevant channel. If this does not help, consider decreasing the concentration of detergent.

Figure 1: During droplet formation, droplets form at the microfluidic junction.



3

Altering droplet size

Oil/aqueous pressure ratios can be altered in order to adjust droplet size. The size of the droplets produced on a microfluidic device is defined in part by the size of the microfluidic junction and in part by the pressure ratio between the oil and the aqueous streams.

- Start in standard run mode, using Dolomite Bio's scRNA-Seq on Nadia protocol as a baseline.
- Once the instrument has reached steady-state, lower the pressure to 250 mbar in the oil channel and 50 mbar in the aqueous channels.
- To **increase** the droplet size, either **increase the aqueous channel pressures** or **decrease the oil pressure**. To **decrease** the droplet size, either **increase the oil pressure** or **decrease the aqueous channel pressures**.
- Take pictures of the junction and droplets and estimate the size of droplets by using the junction (80 µm) as a reference.
- For a more accurate measurement of droplet size, stop the run, pipette some emulsion into a plastic haemocytometer and measure the droplets using the grid as a reference.

Changing temperature and timing

Sample loading temperature

- 5 °C is the standard temperature for sample loading. Increasing the temperature may affect cell viability during sample loading.

Run temperature

- 6 °C is the standard run temperature. Increasing the temperature will reduce reagent viscosity and can affect droplet formation by causing jetting (see section 2 for definition of jetting).

Post-run (lysis) temperature and time

- 22 °C is the standard post-run temperature.
- Increasing the temperature can help improve lysis. The Nadia Innovate can reach a maximum temperature of 40 °C.
- It is also possible to improve lysis by incubating the emulsion for longer. For example, the scRNA-Seq-on-Nadia protocol involves a 10 min post-run incubation step whilst the sNuc-Seq-on-Nadia protocol allows for up to 45 mins incubation.

Adjusting stirring of cells and beads

Cell and bead agitation is crucial for even encapsulation and low doublet rates throughout the run. The stirring speed can be altered depending on bead and cell type in order to achieve optimal results.

Stirring hard beads

- Start in standard run mode, using Dolomite Bio's scRNA-Seq on Nadia protocol as baseline.
- Once in steady-state, gradually increase the stirrer speed until the beads are fully in suspension.
- Choose the minimum stirrer speed that can keep beads fully suspended to avoid bead damage.
- The standard pre-run stirring time for hard beads is 30s. Consider increasing this time to up to 1 min if beads are prone to clumping. It is not recommended to stir beads pre-run for more than 1 min to avoid cell sample degradation.

Stirring soft beads

- 25 rpm is the recommended stirring speed for soft beads, due to their properties making them more fragile than hard beads.
- Keep stirring speed low to maintain a packed bed of beads entering the channel.

Stirring cells

- 75 rpm is the recommended stirring speed for most cell types tested so far.
- For fragile cell types, reduce the stirring speed and check that the cells remain fully suspended.

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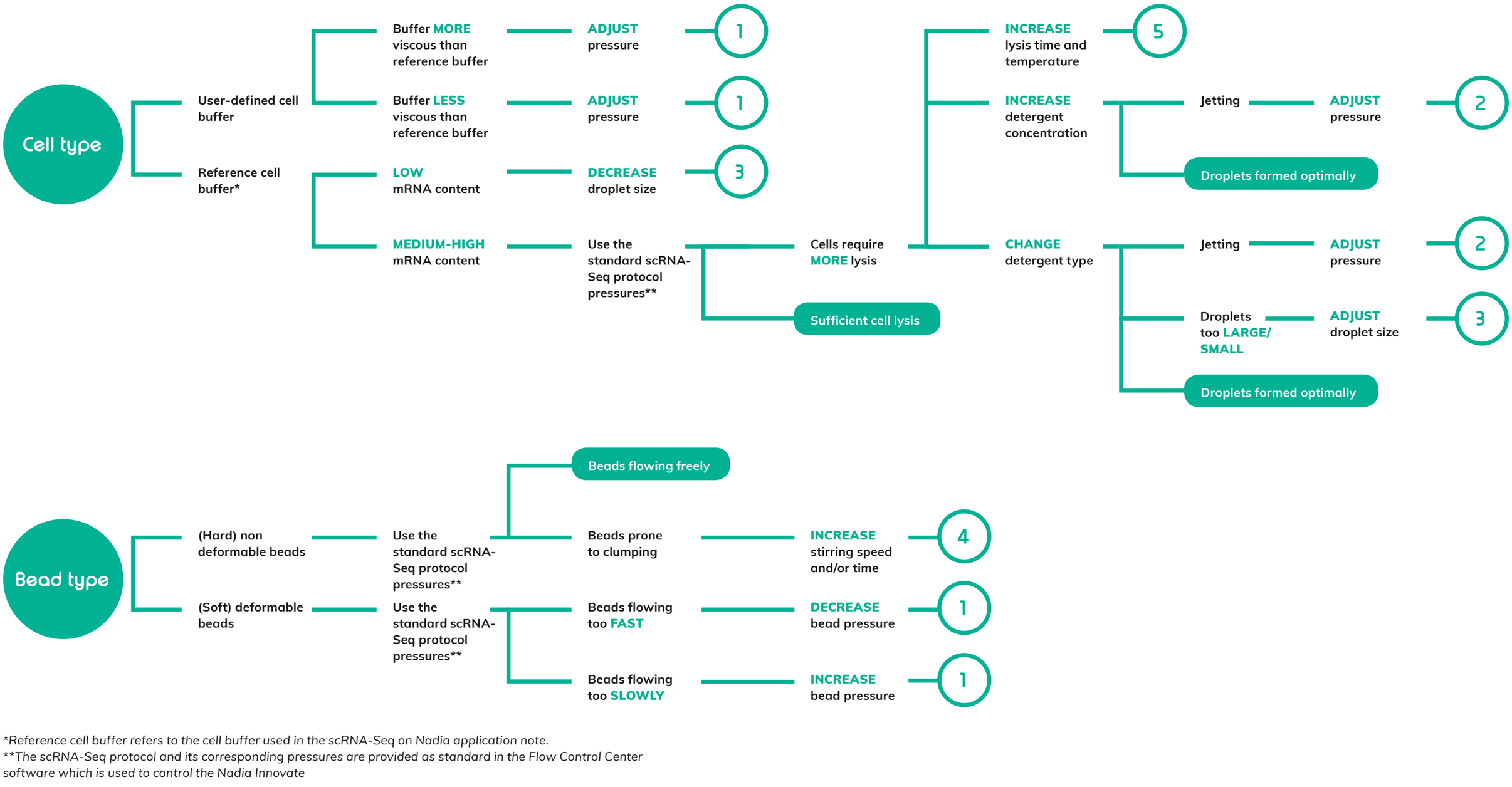
Rapid optimization and protocol development

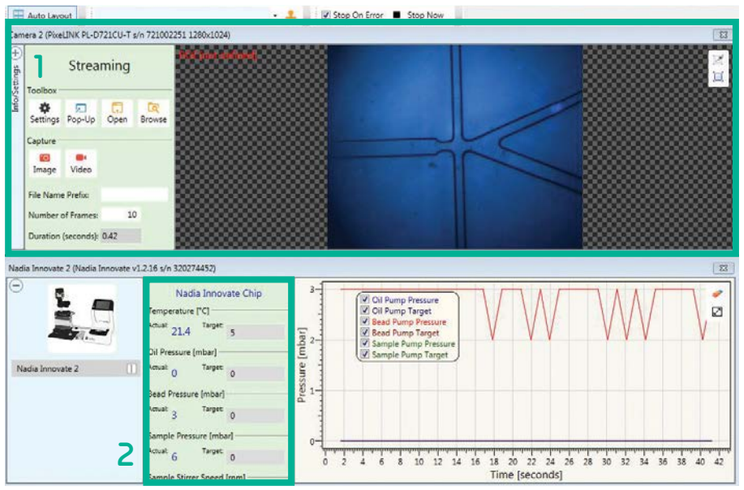
This workflow illustrates the necessary steps to be undertaken when looking to optimize and develop customized single cell protocols. Follow the steps based on cell and bead types used to understand and identify when to change parameters to meet the requirements of your single cell experiment.

> **“The Nadia Innovate is incredibly intuitive and easy to use, and its versatility has allowed us to optimize parameters, giving a better insight into gene expression in each cell.”**
>
> Dr Chris Sibley, Imperial College London



*Reference cell buffer refers to the cell buffer used in the scRNA-Seq on Nadia application note.
**The scRNA-Seq protocol and its corresponding pressures are provided as standard in the Flow Control Center software which is used to control the Nadia Innovate

Case studies	Background	Parameters changed	Details
scRNA-Seq with plant protoplasts	Whilst animal cells can typically be stored and encapsulated in simple buffers such as PBS, plant protoplasts require more viscous buffers with high osmolyte contents to prevent premature cell lysis.	Increased sample pressure Increased oil pressure	For protocol development, sample and oil pressure were adjusted so that droplets could be produced. With this new pressure profile, it was possible to encapsulate plant protoplasts in their compatible buffer with high throughput ready for single cell RNA sequencing.
Agarose encapsulation*	Encapsulating single cells in agarose droplets permits cell growth within a microenvironment for days after encapsulation. Cells encapsulated within hydrogel droplets can be individually subjected to single molecules, drugs, substrates or other cells before being sequenced or sorted.	Increased pressure on bead line Increased temperature to 37 °C Increased stirrer speed	Increasing the pressure in the bead line was required to allow agarose encapsulation. This new pressure was used to flow molten agarose into droplets containing cultured cells. The reservoir stirrer speed was increased to ensure that the agarose remained mixed throughout encapsulation.
sNuc-Seq	Use of smaller droplets when encapsulating nuclei may improve overall mRNA capture efficiency in nuclei.	Increased pressure on oil (700-800 mbar), bead (130-140 mbar) and cell lines (130-140 mbar)	For this protocol, sample and oil pressures were adjusted to produce smaller droplets of about 69 microns.
Deformable beads	Use of deformable beads during encapsulation can result in a higher proportion of droplets containing a bead and consequently a higher cell capture rate.	Adjust pressures on oil (280-320 mbar), bead (155 mbar) and cell lines (105 mbar)	For this protocol, deformable beads, sample and oil pressures were adjusted to achieve a bead capture efficiency of >70%.



The Flow Control Centre software, used to control the Nadia Innovate platform, enables:

1. Real-time visualization of microfluidic junction
2. Adjustment of pressure parameters during a run for dynamic protocol optimization

**Dolomite Bio's agarose application note is available to download on our website.*

Top tips

Cell-related research considerations

A standard scRNA-Seq-on-Nadia run requires 75,000 single cells per sample (300 cells/µl in 250 µl). This number can be reduced by diluting the cells and/or reducing the volume of the cell suspension (100 µl minimum). Using a lower concentration of cells will lower the doublet rate but will also lower the throughput. It is important to strike a balance between the two.

General considerations when handling cells:

- **Difficult to prepare single cell suspensions**
  Consider isolating nuclei instead as proxies for single cells.
- **Cells with low mRNA concentration**
  Decrease the size of droplets. This will increase the effective concentration of mRNA inside the droplets. It is also possible to include an incubation step at the end of the run to further increase mRNA capture.
- **Delicate cells**
  Consider stirring at very low speed.
- **Difficult to lyse cells**
  Increase the time, detergent concentration and/or temperature during the lysis step.

How to avoid blockages

Always filter fluids before loading onto the Nadia Innovate microfluidic chip. Dust and fibres can clog the chip's channels. Ensure that cells or nuclei are individualised and passed through a cell strainer to remove clumps before being loaded onto the Nadia Innovate chip. When using beads that are prone to clumping, ensure that they are resuspended thoroughly before loading.

Bead type and cell capture rate

Non-deformable (hard) beads
In a standard scRNA-Seq on Nadia run, 6,000 cells are captured together with non-deformable (hard) beads. Whilst this translates into a capture rate of only 10%, 6,000 cells in the majority of experiments are sufficient to address relevant biological questions. Furthermore, non-deformable beads can be flowed at high flow rates and are generally more stable than deformable (soft) beads.

Deformable (soft) beads
Whilst deformable (soft) beads can be loaded at higher concentrations, resulting in higher cell capture rates, they are generally more prone to breakage and require flowing at slower speeds. However, specifically when handling small amounts of input materials or precious cells, the use of deformable (soft) beads is recommended.

Changing pressure profiles

It is possible to alter pressures. However, during the priming stage, it is recommended not to change the pressures as these were optimized to avoid blockages at the start of the run.

Buffer components

Avoid using buffers with a pH **lower than 5 or greater than 9.5.**

In addition, some large biological molecules, such as biotin, are prone to crosslinking and forming protein aggregates that can disturb droplet formation in high concentrations. They should therefore be avoided.

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