

Development of custom single-cell assay protocols using the ICELL8 cx system and open architecture



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Abstract

The use of next-generation sequencing in clinical research and applied spaces requires accurate, parallel processing of large numbers of samples and the availability of chemistries that enable robust library preparation from the desired targets. Given the rapid advancements in single-cell and nucleic acid research, one of the challenges is finding an instrument platform that can support such dynamic fields. In this poster, we will present a diversity of single-cell assay workflows developed successfully using the ICELL8® cx Single-Cell System’s nanowell technology, imaging capabilities, and software solutions.

Developing new assays for high-throughput analysis of single cells requires an open and flexible platform. The ICELL8 cx system was designed with these features in mind, and the new ICELL8 cx CELLSTUDIO™ v2.0 Software (CELLSTUDIO Software) provides a simple graphical user interface (GUI) for developers to design, test, and improve their own miniaturized protocols. The types and number of samples that can be tested are configurable, and utilizing multiple reagent source wells allows for multiple reaction conditions to be tested in parallel. Along with high numbers of replicates for each reaction condition, positive and negative controls can be included with each test condition, giving researchers confidence in their results. The ICELL8 cx system is also a high-throughput method to isolate nuclei or single cells of any size—even accommodating cardiomyocytes and organoids—while providing control over the selection of the isolated cells out of all available 5,184 nanowells. Furthermore, the system enables the flexibility to analyze multiple parameters per experiment and uses the power of imaging to distinguish and select desired cells based on viability or phenotype—allowing meaningful conclusions to be drawn from the data. CELLSTUDIO Software and the ICELL8 cx system together provide an ideal system for the development of miniaturized, high-throughput single-cell assays.

Executive summary

Obtaining meaningful results from NGS assays requires researchers to have an unbiased method to analyze large numbers of cells, greater control over the selection of the cells to be processed, and the flexibility to accommodate any cells, reagents, and experimental parameters. The ICELL8 cx system meets these requirements with an integrated and automated platform that lets you conduct your single-cell sequencing projects your way.

You control your experiment with greater:

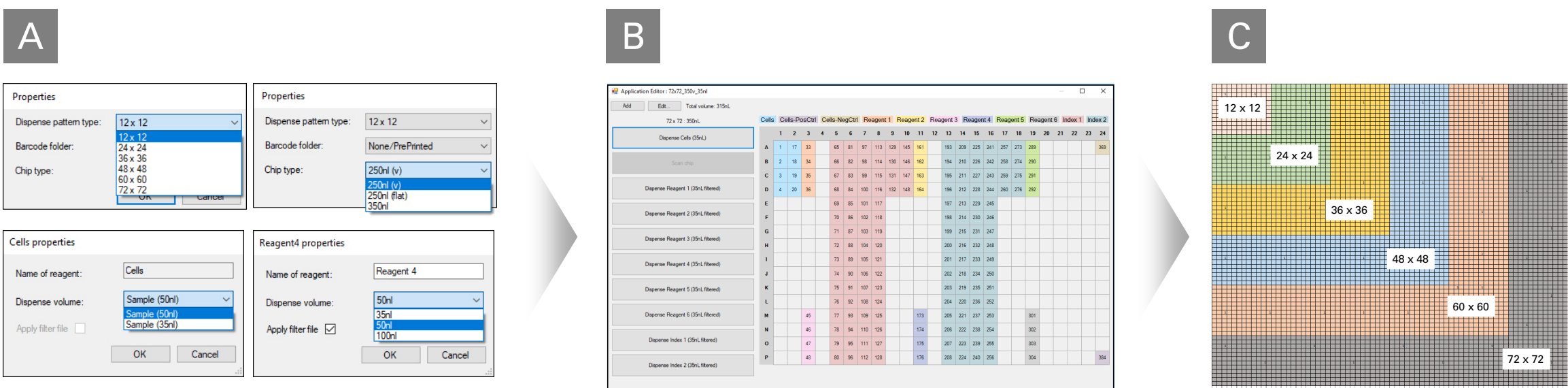
- **Flexibility**—customize your experimental setup with flexible dispensing patterns, and develop applications of your choice or run prevalidated chemistries
- **Confidence**—reduce batch effects by eliminating unnecessary analysis of empty or multicell wells, tie sequencing data to individual cells, and perform replicates

1 An end-to-end solution for your single-cell needs



**Figure 1. ICELL8 cx Single-Cell System.** At Takara Bio, we understand the challenges that come with automating and scaling your single-cell research. To help accelerate your workflow, we have designed the ICELL8 cx system to be an open, flexible platform compatible with trusted reagents and easy-to-use software tools that simplify your experimental workflow.

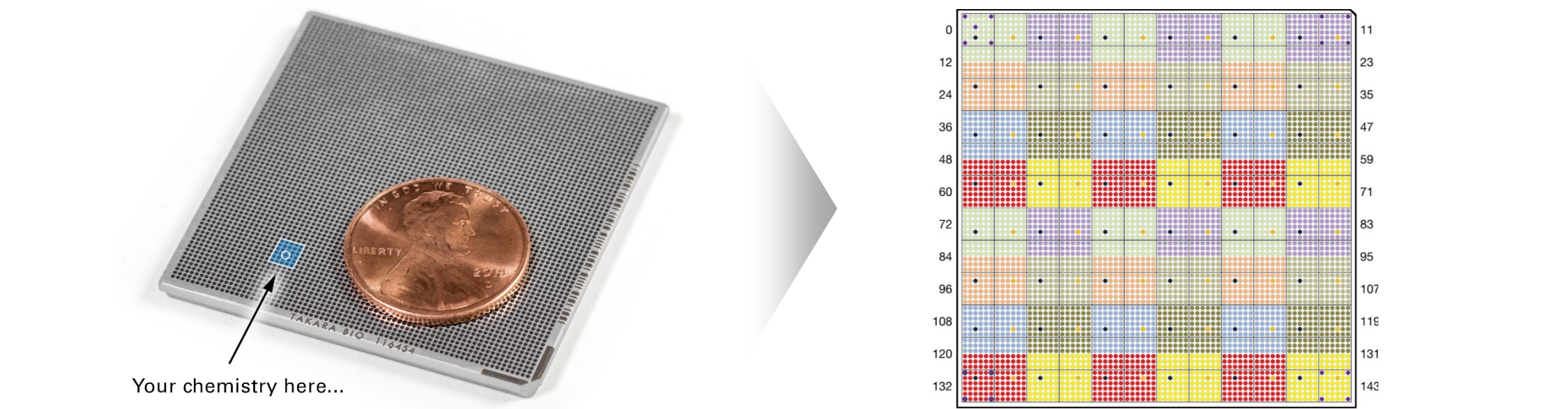
2 Limitless possibilities for creating your NGS assays



**Figure 2. CELLSTUDIO open-architecture software.** The ICELL8 cx system offers the CELLSTUDIO Software application tool, which allows you to miniaturize and automate your single-cell or NGS assays at nanoscale levels in a high-throughput manner. Seamlessly adapt your bench protocol to the ICELL8 cx system by using simple, app-based selections for your experiment’s multistep reagent additions. **Panel A.** Simple GUI allows the user to define dispense patterns, chip types, reagent types, and volumes for each reagent dispense. **Panel B.** The application editor interface shows the dispense menu and the source plate layout. **Panel C.** Flexible dispense pattern for your NGS assay during development. Dispense patterns range from a 12 x 12 layout for 144 samples (including 4 positive and 4 negative controls) to a 72 x 72 layout for 5,184 samples (including 48 positive and 48 negative controls).

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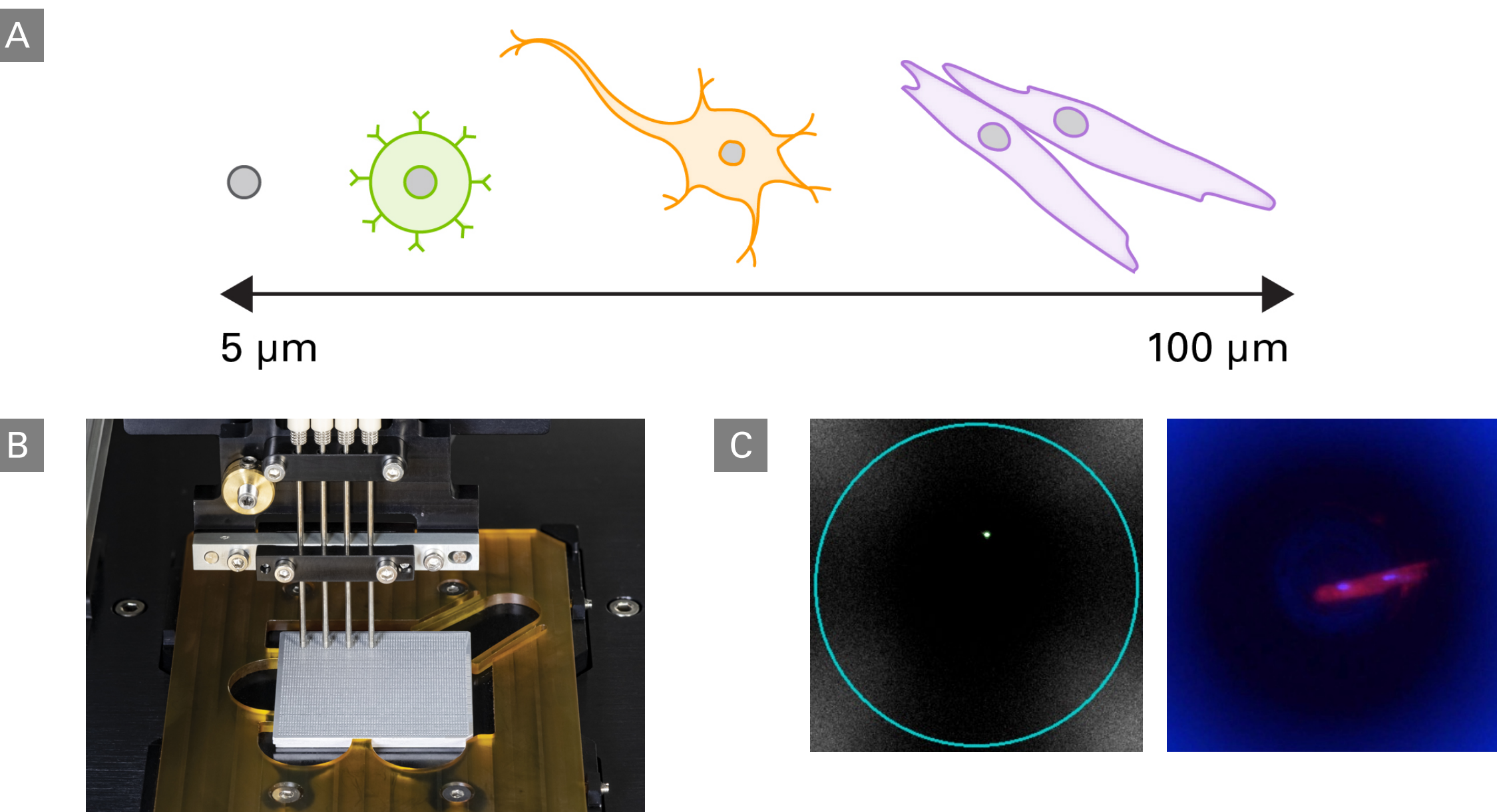
3 Flexibility to explore the questions that matter



- Use smaller reagent volumes with nanoliter-sized reactions
- Achieve multistep additions to your reactions—not typically possible with droplet-based systems
- Process up to 8 samples per chip, perform replicates, and include controls to reduce batch effects

**Figure 3. Nanowell technology.** The power of the ICELL8 cx system starts with unique 5,184-nanowell chips, available with preprinted barcodes for validated chemistries or in blank formats to allow you to develop your own applications. **Left.** Closeup of an ICELL8 cx chip with a penny shown for scale. **Right.** Samples and controls are dispensed in quadrants so that they are distributed across the entire chip.

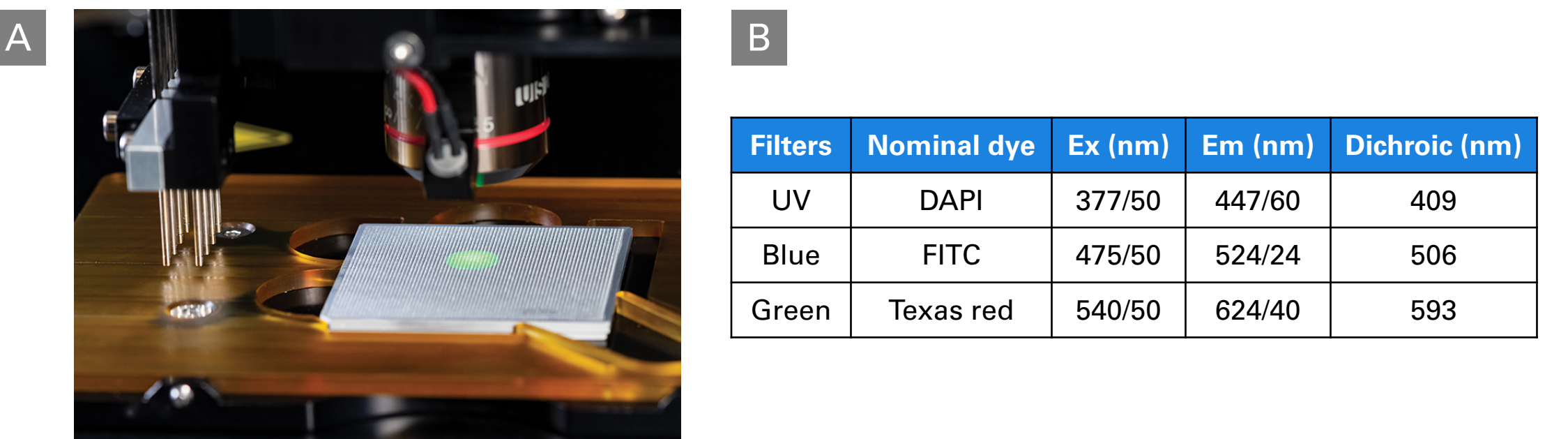
4 Power to handle samples of any size



- Save precious reagents with a minimum dispense volume of 35 nL per well
- Avoid disruption of fragile cells with gentle dispensing
- Dispense samples and controls across the entire chip
- Maintain cell viability with temperature- and humidity-controlled dispensing chamber

**Figure 4. Biological flexibility: applicable to many cell types.** Panel A & B. Cell sizes are just as varied as the functions they perform. The ICELL8 cx nanodispenser takes advantage of a large-bore nozzle for dispensing and Poisson distribution, allowing users to isolate samples of any size, from nuclei to cardiomyocytes, and even spheroids and organoids. Refer to [takarabio.com/celltypes](https://takarabio.com/celltypes) for a list of cells that have been demonstrated to date. **Panel C.** Left. Image of a single nucleus in a well. Right. Dispensed adult cardiomyocytes stained with Hoechst and CellTracker Red dyes. Data kindly provided by Dr. Stefan Günther, Max Planck Institute.

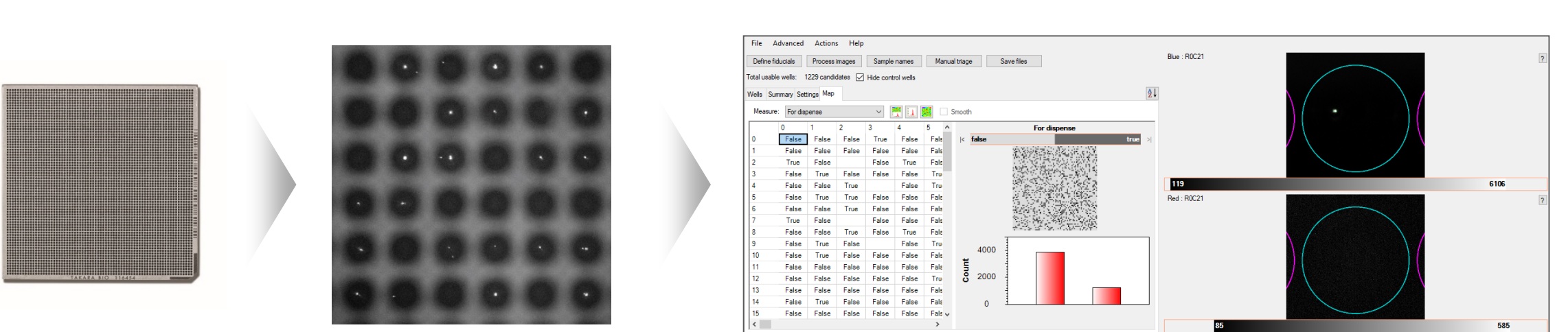
5 Confidence in your data with cell visualization



- Image all cells in ~15 minutes
- Tie sequencing data back to individual wells
- Accommodate many cell-staining dyes and surface-labeling markers

**Figure 5: Imaging system.** Panel A. The 8-channel controlled dispenser takes advantage of a large-bore nozzle for dispensing, allowing users to isolate cells of any size, including nuclei, cardiomyocytes, spheroids, and more. The unbiased cell isolation yields 1,000–1,800 single cells per chip, and dispensing is complete in just 15 minutes. **Panel B.** The ICELL8 cx system features built-in, automated imaging capabilities that allow differentiation of wells with single cells from those with multiple or no cells. The available three-color filter can be used in conjunction with cellular dyes for live/dead analysis or phenotypic selection.

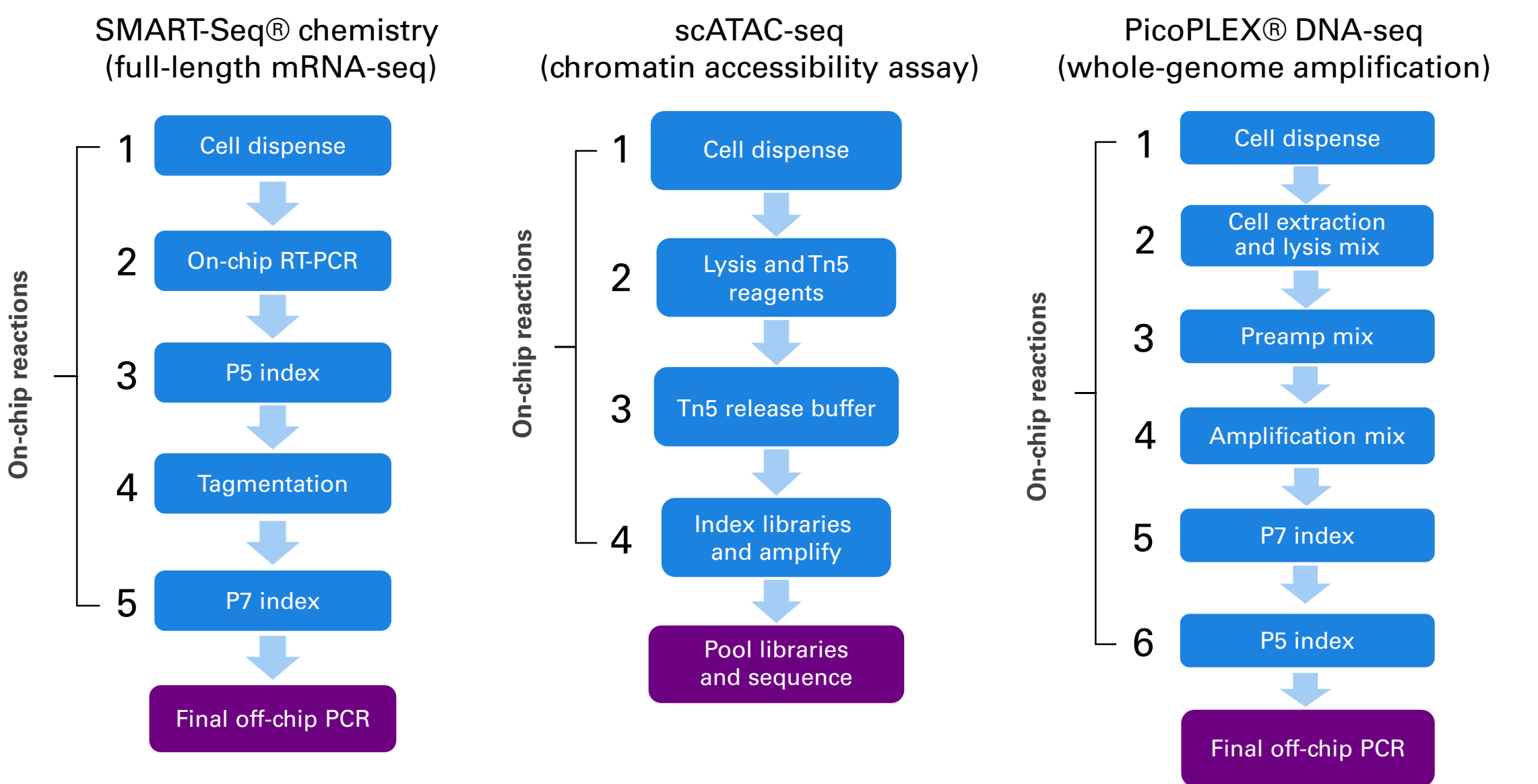
6 Greater control over your downstream processing



- Identify true, live, single cells and reject dead cells or doublets automatically
- Downselect wells to get the most amount of reads per sample for your sequencing platform
- Tune imaging and analysis settings to meet your specific parameters

**Figure 6. ICELL8 cx CellSelect® v2.0 Software.** The integrated, proprietary CellSelect Software analyzes well images and provides the ability to proceed only with the wells you choose, such as eliminating the noise from multiple-cell-containing or empty wells.

7 Innovate new applications using our open format



**Figure 7. Custom applications.** ICELL8 technology was designed to be open and flexible so that researchers could develop new single-cell sequencing applications. Several researchers have already designed custom single-cell epigenomic assays, nuclei sequencing strategies, and beyond.

Customer-developed applications

- Gao, R. et al. Nanogrid **single-nucleus RNA sequencing** reveals phenotypic diversity in breast cancer. *Nat. Commun.* 8, 228 (2017).
- Hochgerner, H. et al. **STRT-seq-2i**: dual-index 5' single cell and nucleus RNA-seq on an addressable microwell array. *Sci. Rep.* 7, 16327 (2017).
- Kaya-Okur, H. S. et al. **CUT&Tag for efficient epigenomic profiling** of small samples and single cells. *Nat. Commun.* 10, 1930 (2019).
- Kim, C. et al. **Chemoresistance evolution** in triple-negative breast cancer delineated by single-cell sequencing. *Cell* 173, 879–893.e13 (2018).
- Kim, S. et al. Generation, transcriptome profiling, and functional validation of cone-rich human retinal **organoids**. *Proc. Natl. Acad. Sci.* 116, 10824–10833 (2019).
- Krieger, T. G. et al. Modeling glioblastoma invasion using human brain **organoids** and single-cell transcriptomics. *bioRxiv* 1–13 (2019). doi:10.1101/630202
- Mezger, A. et al. **High-throughput chromatin accessibility profiling** at single-cell resolution. *Nat. Commun.* 9, (2018).
- Schoger, E. et al. **CRISPR-mediated activation** of endogenous gene expression in the postnatal heart. *Circ. Res.* 126, 6–24 (2020).
- Shvartsman, M. et al. Single-cell atlas of major haematopoietic tissues sheds light on blood cell formation from embryonic endothelium. *bioRxiv* 774547 (2019).
- Tirier, S. M. et al. **Pheno-seq** – linking visual features and gene expression in **3D cell culture systems**. *Sci. Rep.* 9, (2019).
- Wu, L. et al. Full-length single-cell RNA-seq applied to a viral human cancer: applications to **HPV expression and splicing analysis** in HeLa S3 cells. *Gigascience* 4, 51 (2015)
- Yekelchik, M., Guenther, S., Preussner, J. & Braun, T. **Mono- and multi-nucleated ventricular cardiomyocytes** constitute a transcriptionally homogenous cell population. *Basic Res. Cardiol.* 114, (2019).