Sony Spectral Cell Analyzer ID7000

Sample acquisition, spectral unmixing, and shutdown

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Adapted from: Surace Lab SOP #001 (Sony ID7000), University Hospital Bonn (v1; 17/08/2023; revised 18/08/2023).

Purpose

Provide a standardized workflow to operate the **Sony Spectral Cell Analyzer ID7000** for spectral flow cytometry, including:

- Instrument startup and (when required) daily QC
- Experiment setup and instrument settings
- Acquisition of single-stain controls and spectral unmixing
- Acquisition of samples
- Export of FCS files and data transfer
- Cleaning/decontamination and shutdown

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Scope

This protocol applies to users performing flow cytometry analyses on the **Sony Spectral Cell Analyzer ID7000** located at the **Flow Cytometry Core Facility (FCCF), BMZII, University Hospital Bonn**.

It covers operation in **Normal mode** and assumes users have completed FCCF induction/training and have appropriate authorization to use the instrument.

Hazards and safety

Hazards

⚠ Chemical hazards

This workflow may involve exposure to the following chemicals (toxic/irritant). Consult local MSDS and FCCF safety guidance before use.

- **ROTI®Histofix 4%** (4% formaldehyde; Carl Roth; Cat. No. P087.1)
- **Bleach** (sodium hypochlorite, NaOCl)
- **Mucocit® T** (Schülke & Mayr GmbH)

⚠ Biological hazards

- Samples may be classified as **S2**.
- **S2 samples must be fixed prior to acquisition** (e.g., ROTI®Histofix 4%, ICS, 4% PFA), according to FCCF and institutional biosafety rules.

Controls

- Wear full PPE (see below).
- Handle chemical reagents in accordance with MSDS and facility guidance.
- Follow FCCF instructions for waste handling and decontamination.

PPE

- Long-sleeved lab coat
- Enclosed footwear
- Gloves
- Additional PPE as required by sample type and local risk assessment

Reagents, consumables, equipment, and buffers

Reagents

Item	Manufacturer	Cat. No.	Location/notes
AlignCheck Particles	Sony Biotechnology	AE700510	FCCF fridge; in foil packet
8 Peak Beads	Sony Biotechnology	AE700522	FCCF fridge; in foil packet

Consumables

- FACS tubes (Sarstedt; Cat. No. 55.1579) **or** 96-well U-bottom plates (TPP®; Cat. No. 92697)
- Aluminum foil

Equipment

- Sony Spectral Cell Analyzer ID7000 (FCCF, BMZII)
- ID7000 computer + ID7000 software
- If running samples in tubes:
  - FACS tube rack
  - 24-position 5 mL tube rack (Sony Biotechnology; stored in FCCF fridge)

Buffers and facility liquids

Use	Name	Notes / location
Sheath	MilliQ water	Used to fill sheath tank
Clean	Bleach	Prepared by FCCF; white squeeze bottles labelled “Bleach”
Rinse	Rinse solution	Prepared by FCCF; blue squeeze bottles
Running buffer	FACS Flow	Prepared by FCCF; dispenser tanks next to sink

General notes

- Use of the Sony ID7000 is subject to **FCCF induction and approval** (FCCF contact person noted in the source SOP: Andreas Dolf). Training can be booked on PPMS.
- FCCF recommends **bead-based single stains** (e.g., UltraComp eBeads Plus), including for **fixable amine-reactive viability dyes** (e.g., ArC Amine-reactive beads).
  - For ArC viability dye single stains: include a matching tube of **unstained ArC beads**.

- Use an **unstained single-cell suspension** (from your tissue of interest) to identify and unmix **autofluorescence** where relevant.
  - Reference: Wanner et al., 2022 (Frontiers in Bioengineering and Biotechnology; doi: 10.3389/fbioe.2022.827987).

Procedure

A. Startup and (when required) daily QC

> **When to run daily QC**
>
> On weekdays, FCCF staff typically perform daily QC by **09:00**. Run the daily QC yourself if: - you start **before** FCCF operating hours, or - you use the machine **on weekends**.

1. Check the **left side** of the instrument and ensure **both black switches** are on.
2. Check **Waste** and **Sheath** tank levels. If necessary:
   - Empty waste into sink, then add **2 pumps of Mucocit® T** to the empty waste tank.
   - Refill **Sheath** tank with **MilliQ water**.
3. Press the **Power** button on the front of the instrument (green light should turn on).
4. Turn on the computer.
5. Log in with your FCCF account.
6. Start the **ID7000 software** (desktop icon).
7. Log into the software.
   - If using a shared lab account containing saved controls, log into that account (as applicable).
8. Confirm the ID7000 connects to the computer (bottom right of software). Wait a couple of minutes.
9. The **Priming Wizard** should open automatically. Click **Start**.
10. While priming runs, prepare QC beads in FACS tubes:
    - **AlignCheck beads:** 450 µL FACS Flow + 2 drops beads
    - **Sony 8-peak beads:** 350 µL FACS Flow + 1 drop beads
11. When priming is complete, close the QC window if it pops up.
12. Click **Start Daily and Performance QC**.
13. Open the instrument door.
14. Remove **Clean** and **Rinse** tubes from the tube station.
15. Place **AlignCheck** and **8-peak** bead tubes into the tube station in the order shown in the QC window.
16. Close the door and follow prompts to run QC.

> **If QC fails due to pressure**
>
> A pressure-related error can cause QC failure. Re-check **Waste** and **Sheath** tank levels and correct if needed, then re-run QC.

B. Experiment setup

1. After QC passes, go to **Experiment** tab.
2. Click **Experiment designer**.
3. Confirm required laser lines are enabled (checkmarks). Enable if needed:
    - 355 nm
    - 405 nm
    - 488 nm
    - 561 nm
    - 637 nm
4. Add fluorochromes (e.g., FITC, APC, PE) by typing and clicking **Add**.

> **i** Order of fluorochromes
>
> - If acquiring single stains in **tubes**, fluorochrome add-order does not matter.
> - If acquiring single stains in a **plate**, match fluorochrome order to the plate layout.
> - If using previously saved single-stain controls from the library and no new controls are needed, you may skip Section C.

5. Fill in metadata (Marker, Species, Clone, Lot number, etc.).
6. Click **Next** until **Instrument Settings** step.
7. Click **Import** and import:
    - `Desktop -> Import Master Setting -> SONY_Master Settings normal.xml`
8. Click **Next** and select acquisition order: **From left-to-right**.
9. Click **Next** and complete experiment details.
10. Click **Create Experiment**.
11. Under **Acquisition** tab, click **Layout Editor** (Plate section).
12. Hold **Ctrl** and select the unassigned positions corresponding to the number of samples.
13. Set **Sample Group** to `Sample Group - 1`.
14. Optionally check **Auto acquisition target**.
15. Name each sample position under **Sample Information**.
16. Close Layout Editor.

C. Acquire single stains and perform unmixing

C1. Load single stains

1. If using tubes:
    - Retrieve the **24-position 5 mL tube rack** from FCCF fridge.
    - The **notch** marks **A1**.
2. Arrange single-stain tubes (or plate) in the order shown in the software layout.

> **i** ArC beads for fixable viability dyes
>
> If using ArC beads stained with a fixable amine-reactive viability dye, also add **unstained ArC beads** as a separate tube. In the software: - Acquisition -> Layout Editor -> select tube position -

Name it (e.g., “Unstained ArC beads”) - Under Sample Settings: - Sample Group: **Single Positive Controls** - Check **Auto acquisition target**

3. Open door, orient rack/plate with **A1 notch** at bottom left, place on AutoSampler.
4. Close door and click **Load**.
    - If “Cooling sample stage is in progress” appears, click **Yes**.

C2. Instrument settings for single stains (example values)

1. Open **Instrument Settings**.
2. Under **Detector and Threshold**:
    - FSC Gain = **17** (example for fixed/permeabilized PBMCs)
    - SSC Voltage (%) = **37.5** (example for fixed/permeabilized PBMCs)
3. Under **Flow Control**:
    - Sample Flow Rate = **3.0**

> **Adjust for your sample type**
>
> The FSC/SSC and flow rate values above are examples given for fixed/permeabilized PBMCs. Adjust for other cell types and staining conditions.

C3. Stopping condition for single stains

1. Open **Stopping Condition**.
2. Set **Total Events = 5,000** (single stains).
3. Click **Sync stopping conditions** to apply to all.

C4. Preview and gate setup

1. Preview each single stain using **Preview**.
2. Adjust gates (“A” and “Positive”) as needed.

> **Gate and scale consistency**
>
> - Apply the same “A” gate to all single stains using **Sync Scale and Gate**.
> - The “Positive” gate should be tight and placed on the highest-intensity events.

> **Off-scale events and voltage changes**
>
> If a conjugate is off-scale, reducing PMT voltage can be used to complete acquisition, but the preferred long-term fix is to **reduce antibody concentration** in future experiments.

3. Click **Stop** to move to the next sample.
    - If it does not advance: right-click tube position -> **Set Current Position**.

C5. Record single stains

1. Right-click the first tube (usually "Unstained") -> **Set Current Position**.
2. Acquire single stains:
    - Manual: **Acquire**
    - Automated: **Auto Acquire** (requires Auto acquisition targets)

C6. Unmixing

1. Go to **Unmixing** tab.
2. Click **Unmixing Settings**.
3. If you added an unstained ArC bead control as the negative gate for the live/dead single stain, confirm the negative reference is correctly assigned.
4. Click **Calculate**.

> **i** Saturation warnings during unmixing
>
> If prompted that saturation of x% is observed: - Small % (e.g., <0.1%): Continue - Large %: the single stain is too bright - Re-run single stain with reduced voltage (Instrument Settings -> Detector & Threshold -> PMT Voltage (%) -> Synchronous Voltage Adjustment) - Preferably: plan to reduce antibody concentration in future experiments

5. When prompted to apply spectral references to other sample groups with the same laser settings, click **Apply**.
6. Confirm the **Unmixing** toggle (top right) is **ON** (blue).

D. Acquire samples

1. If single stains were already acquired and you are switching racks/plates:
    - Click **Unload** and wait for "Plate Unloaded"
    - Open door, remove rack/plate, close door
2. Place your sample tubes in the rack according to your Layout Editor assignments.
3. Open door, load rack onto AutoSampler, close door, click **Load**.
4. Confirm **Unmixing** toggle is **ON** (blue).
5. Set/confirm instrument settings (adjust as needed):
    - Detector & Threshold: FSC Gain and SSC Voltage appropriate for sample
    - Flow Control:
        - Sample Flow Rate = **3.0** for previewing ~5 x 10^5 cells in 100 µL
        - Sample Flow Rate = **8.0** for recording ~5 x 10^5 cells in 100 µL
    - Agitation: enable, pattern **Cyclic**, mode **Normal**
    - Event Check: **On**
6. Set stopping condition (events or time) according to your experimental requirements.
7. Draw/confirm gating strategy in the worksheet area.
8. Select the sample to run first: right-click -> **Set Current Position**.
9. Click **Preview** (green arrow) to verify signals are on scale.
10. Click **Record** (red circle) to record selected sample, or **Auto Acquire** to run all auto targets.

> ⚠ Avoid running samples dry
>
> If you need time to adjust axes or gates, stop acquisition to prevent samples running dry.

E. Export and transfer data

> ⚠ No external drives
>
> Do not plug external devices (e.g., USB drives) into FCCF computers. Transfer data via approved methods (e.g., BTC cloud), per facility policy.

1. Under the Experiment pane, click the experiment name (top item in hierarchy).
2. Right-click -> **Export to FCS file**.
3. Export settings:
   - Under "Pulse Type (for fluorescence)": uncheck **Height** and **Width**
   - Ensure **FCS version = 3.1**
   - Set export location under **Save in**
4. Click **Export** and wait for export progress to complete.
5. Confirm export has started/completed via the popup and/or log file in output folder.
6. Transfer exported files to your BTC cloud account (or other approved location).

F. Cleaning and shutdown

F1. If another user is booked after you

1. Click **Unload**.
2. Remove rack/plate from AutoSampler.
3. Go to **Cytometer** tab -> **Decontamination**.
4. Select **Bleach Cleaning and Rinse** and follow prompts to completion.
5. Remove samples, return tube rack to FCCF fridge.
6. Close ID7000 software.
7. Ensure:
   - Waste tank is empty
   - Sheath tank is full for next user

> ℹ Red error light after closing software
>
> After the software closes, a red "Error" light on the ID7000 is normal and indicates the instrument is not connected to the computer.

F2. If you are the last user for the day

1. Click **Unload**.
2. Remove rack/plate from AutoSampler.
3. Go to **Cytometer** tab -> **Hardware Shutdown**.

4. Select **Bleach Cleaning and Rinse** and follow prompts to complete decontamination and shutdown.
5. Remove samples, return tube rack to FCCF fridge.
6. After shutdown completes and cytometer switches off automatically, close the software.
7. Ensure waste is empty and sheath is full for next user.

Troubleshooting

- **QC fails due to pressure:** re-check waste/sheath levels and re-run QC.
- **Single stain saturation during unmixing:** re-run with reduced PMT voltage short-term; reduce antibody concentration long-term.
- **Software does not advance tube position:** right-click the tube position -> **Set Current Position**.

References and links

- Booking (PPMS): `https://ppms.eu/uni-bonn/login/?pf=2`
- Sony ID7000 Spectral Viewer: `https://www.sonybiotechnology.com/us/instruments/id7000-spectral-ce`
- Autofluorescence reference: Wanner et al., 2022. Front Bioeng Biotechnol. doi: `10.3389/fbioe.2022.827987`

Version history

Version	Date	Author	Changes
v1.0	2025-11-21	Dillon Corvino	Converted Sony ID7000 SOP into Abdullah Lab Quarto protocol standard.