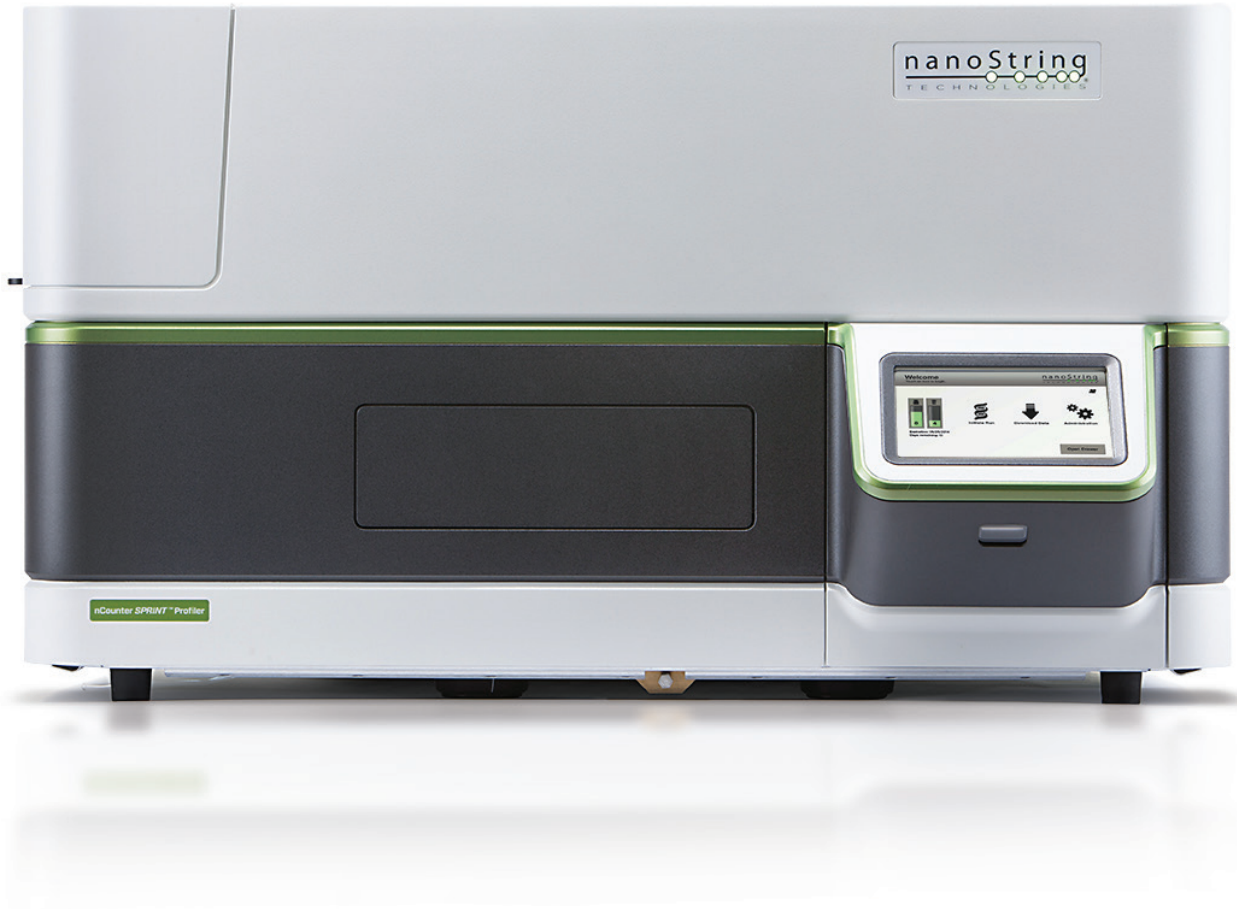
nCounter *SPRINT* | THE **REVOLUTION** HAS BEGUN



Frequently Asked Questions

Differences between the nCounter® Analysis System and the nCounter *SPRINT* Profiler.

1. This table (continued on the next page) compares some features of each instrument.

Feature	nCounter Analysis System[1]	nCounter *SPRINT* Profiler
Ability to Run Prosigna® Breast Cancer Prognostic Gene Signature Assay	Yes, with upgrade to nCounter Dx with *FLEX* configuration[2].	No. Research Use Only. Not for use in diagnostic procedures.
Hands-on Time	15 minutes Setup times: ▪ Set up sample hybridization: 5 minutes ▪ Initiate run on Prep Station: 5 minutes ▪ Initiate run on Digital Analyzer: 5 minutes	10 minutes Setup times: ▪ Set up sample hybridization: 5 minutes ▪ Initiate run: 5 minutes
Instrument Procedure	Two steps: 1. Purify hybridized samples with the Prep Station. 2. Scan hybridized samples with the Digital Analyzer.	One step: 1. Purify and scan hybridized samples.
Run Modes	Multiple options	One mode
Run Time	Prep Station ▪ Standard: ~2.5 hours ▪ High Sensitivity: ~3 hours Digital Analyzer[3] ▪ Low: ~0.5 hour ▪ Medium: ~1.25 hours ▪ High: ~2.7 hours ▪ Max: ~5 hours	~6 hours
Runs Per Day (10-hour Day)	4 Runs (Using one Prep Station in standard mode and one Digital Analyzer in any mode.)	2 Runs (Using one nCounter *SPRINT* Profiler.)
Sample Processing	Uses automated pipette tips and reagent Prep Plate cassette.	Uses automated microfluidic cartridge.
Sample Input Amount	RNA ▪ 50 ng (using high sensitivity protocol) DNA ▪ 300 ng RNA:Protein ▪ 400–5,000 cell equivalent lysates	RNA ▪ 25 ng for > 400 target plex ▪ 50 ng for < 400 target plex DNA ▪ 150 ng RNA:Protein ▪ Optimal spot density = 0.8 to 1.1 ▪ Optimal sample inputs may vary with experimental conditions.
Input Volumes	▪ Min: 15 µL ▪ Max: 120 µL	▪ Min: 25 µL ▪ Max: 35 µL
Reporter Stretching	Electro-stretch (Electrophoretic forces.)	Flow-stretch (Mechanical forces by flowing buffer.)
Light Source	4-channel LED light pipe hybrid.	3-channel LED light pipe hybrid.
Slide Binding	Homogenous	Gradient
Scanning Area	Four modes: ▪ Low: 0.9 mm^2 ▪ Medium: 3.6 mm^2 ▪ High: 10.0 mm^2 ▪ Max: 19.6 mm^2	One mode: 10.0 mm^2
Linear Dynamic Range	5 logs: 7 x 10^5 total counts	5 logs: 6 x 10^5 total counts

1. Specifications may not be applicable to Prosigna. See www.prosigna.com.
2. The nCounter Analysis System is also available for research use only. Not for use in diagnostic procedures.
3. Load up to 6 cartridges for unattended operation overnight.

(Continued.)

Feature	nCounter Analysis System	nCounter *SPRINT* Profiler
Cartridge Re-scan Supported	Yes.	Coming soon.
Control Center	Not included.	Set up run and access from desktop.
iPhone® App	No.	Monitor run from anywhere. Coming soon.
Number of Instruments Per System	Two instruments: ▪ nCounter Prep Station ▪ nCounter Digital Analyzer	One instrument: ▪ nCounter *SPRINT* Profiler
Linear Bench Space	145 cm (includes 12 cm between instruments to access on/off switch)	91 cm
Dimensions (W x D x H)	Prep Station: 67 x 89 x 63 cm Digital Analyzer: 66 x 66 x 48 cm	Base only: 91 x 64 x 53 cm Including touchscreen: 91 x 76 x 53 cm
Weight	Prep Station: 120 kg Digital Analyzer: 57 kg	100 kg
Number of Electrical Outlets Required	Two outlets: 100–240 VAC, 50/60 Hz, 600 VA	One outlet: 100–240 VAC, 50/60 Hz, 600 VA
Master Kit Configuration	Master Kit is provided as one SKU: ▪ Prep plate (contains processing reagents) ▪ Plastic consumables ▪ nCounter Cartridge	Master Kit is provided as two SKUs: ▪ nCounter *SPRINT* Reagent Pack ▪ nCounter *SPRINT* Cartridge
Plastic Consumables for Hybridization Step	Notched strip tube is included in the Master Kit.	Not included. Use plastic consumables recommended by your thermal cycler manufacturer.

2. Are there any improvements in sensitivity vs. previous instruments?

The nCounter *SPRINT* Profiler is designed to use lower sample input amounts. However, it has the same limit of detection (LOD) as the nCounter Analysis System (15 zeptomole spike-in control equivalent to 10,000 RNA copies).

3. How is scanning different with the new system?

There are two primary differences between the nCounter *SPRINT* Profiler and nCounter Analysis System.

First, the reporter probes are stretched differently prior to imaging. The nCounter Analysis System uses electrophoretic stretching to position the reporters for imaging, and the nCounter *SPRINT* Profiler uses microfluidic flow.

Second, different light sources are used for the optical microscope that scans the reporter probes. The nCounter Analysis System uses a four-channel illumination system. The nCounter *SPRINT* Profiler uses three-channel illumination to read each of the four colors that make up the reporter. Green and yellow are excited by the same light source, and the green is spectrally resolved from the yellow.

4. Why is throughput so different between the two systems?

The nCounter *SPRINT* Profiler is a single instrument designed for reduced hands-on time as the cartridge proceeds from purification to imaging without user intervention. The nCounter Analysis System consists of two independent instruments, the Prep Station and the Digital Analyzer. These instruments can be run independently but require user intervention when transferring the cartridge after purification adn before imaging. Additionally, the nCounter Digital Analyzer was designed to hold up to six cartridges for unattended operation overnight, which permits increased throughput.

Installation, Training, and Maintenance

5. How long does it take to install the nCounter *SPRINT* Profiler?

It will take a service engineer approximately two days to install the instrument.

6. Can I move the instrument after it is installed?

Please contact NanoString Support to arrange for a service engineer to move your instrument, as significant damage may be caused by moving it without NanoString's assistance. Moving the instrument by other means will void the warranty.

7. Why should I network the nCounter *SPRINT* Profiler?

Connecting the instrument to a local area network (LAN) enables users to access the Control Center to remotely set up runs, get data, and check run status. In addition, networking will make it easier to send log files to NanoString Support for troubleshooting.

8. How do I network the nCounter *SPRINT* Profiler?

Connect the instrument to a local area network (LAN) using a CAT5e Ethernet cable. Refer to the user manual when configuring the system. For assitance, please contact at IT professional at your institution.

9. If sample processing is interrupted, am I at risk of complete sample and data loss?

Yes, all data will be lost if an interruption occurs before scanning.

10. How should I protect my runs from power loss?

Power loss may result in loss of data. NanoString recommends connecting the nCounter *SPRINT* Profiler into an emergency power outlet. If one is not available, NanoString recommends using an un-interruptible power supply (UPS; part number UPS-USDM-110).

11. Is training included with the purchase of an instrument?

Yes, a field application scientist will provide on-site training for the system and applications.

12. How long does a training session take?

Training by a field application scientist will typically take two days. The time depends on the total number of applications, as each requires instrument processing time. Plan to be available for the following approximate amounts of time: 2 hours for technology and instrumentation training, 1–3 hours for lab work, and 1.5 hours for data analysis. (Add one hour for each additional application.)

13. Can I trust inexperienced users to walk up to the system and use it without damaging the components?

Training is recommended, however the nCounter *SPRINT* Profiler contains video tutorials and other on-screen prompts that list step-by-step instructions for use.

14. How long is the manufacturer's warranty?

The manufacturer's warranty is one year. Service contracts are recommended and can be purchased for periods of 12 to 48 months.

15. What is the recommended routine maintenance on the system?

Additional maintenance tasks may need to be performed as necessary to ensure proper operation of the instrument. Planned maintenance is included in service contracts. Refer to user manual for additional details.

Using the nCounter *SPRINT* Profiler

16. Should I turn the machine off when not in use?

NanoString recommends leaving the instrument turned on for convenience. If the machine is on and not in use, the system will draw 145 volt-amperes. This is equivalent to leaving a PC idle without the monitor plugged in. Refer to the manual for instructions if you prefer to turn off the instrument.

17. Should I leave the reagents connected to the instrument when not in use?

NanoString recommends leaving reagents connected to the instrument. If the instrument is stored without reagents, the operator must perform dry down and long-term storage procedures.

18. Which nCounter panels and applications can be run using the nCounter *SPRINT* Profiler?

nCounter CodeSets and Panels and nCounter Elements™ TagSets can be used with the nCounter *SPRINT* Profiler. These products are for research use only not for use in diagnostic procedures.

19. Is my existing CodeSet compatible with nCounter *SPRINT* Profiler?

Yes, existing CodeSetes are compatible. However, you must use an nCounter *SPRINT* Cartridge and Reagent Pack for purification and imaging.

20. What are the instrument running costs?

The nCounter *SPRINT* Profiler running costs are comparable to previous nCounter systems. Running costs depend on the application and project size. Contact your Regional Account Manager or sales representative to discuss your project.

21. How many samples can I run?

The nCounter *SPRINT* Profiler processes 12 flow cells per run. This means that you can run up to 12 samples without sample plexing or up to 24 samples when plexing two samples per lane.

22. How do I migrate an assay from the nCounter Analysis System to the nCounter *SPRINT* Profiler?

Adjust the hybridization sample input according to the recommended guidelines in the appropriate assay user manual. After hybridization is complete, ensure the sample volume is between 25-35 µL by adding additional RNase-free water, if needed.

23. What is the data correlation between the nCounter Analysis System and the nCounter *SPRINT* Profiler?

Normalized runs from the nCounter Analysis System and the nCounter *SPRINT* Profiler correlate very closely. The best practice is to run a control sample using each instrument and utilize typical controls (housekeeping genes, ERCCs, etc.) to normalize from run to run and instrument to instrument. It is important to note that sample input recommendations for the nCounter *SPRINT* Profiler are lower than those for the nCounter Analysis System, so adjust sample input appropriately.

24. Can I use a multi-channel pipette to load the nCounter *SPRINT* Cartridge?

Yes. It is possible to load 6 samples at one time (using six pipette tips) and perform this action twice to load all 12 samples. However, NanoString recommends using a single-channel pipette to ensure consistent sample loading.

NanoString confirmed that the following multi-channel pipettes and pipette tips are dimensionally compatible. However, their use may make it more difficult to ensure consistent sample loading.

- Pipet-Lite Multi Pipette L8-50XLS+ (5-50 µl volume)
- Pipet-Lite Multi Pipette L8-200XLS+ (20-200 µl volume)
- RT-L200F 200 µl pipette tips

25. I've seen more saturation problems using the nCounter *SPRINT* Profiler than I previously did using the nCounter Analysis System. Does the new system have greater dynamic range for accommodating higher counts?

No, NanoString recommends optimizing sample input to achieve a spot density range of 0.8 to 1.1. For additional information, refer to the tech note *Strategies for Successful Gene Expression Assays*.

26. Why should there be an air bubble in the cartridge?

It is important to depress the pipette plunger to the second stop and create an air bubble behind the sample. This facilitates consistent sample processing. Many people only depress the plunger to the first stop, which is enough to eject the sample but does not create an air bubble. Note that these air bubbles do not need to be the same size (the instrument will correct most variation).

Always pull the pipette away from the sample loading port before releasing the plunger; do not release the plunger immediately. The microfluidic channels are a contained system, and releasing the plunger too soon will withdraw the sample back into the tip. This can be useful if you make an error and need to load the sample again, but it is not good practice.

27. What do I do if I scan my samples using the wrong Reporter Library File (RLF)?

Use the nCounter *SPRINT* Profiler (if networked) or the Control Center to send an email containing the run log files to technical support. Be sure to include the correct RLF name in the email.

28. What plastic consumables should I use for sample hybridization?

To ensure accurate sample temperature and to minimize evaporation risk, NanoString advises using plastic consumables that are recommended by the manufacturer or your thermal cycler.

29. What are the minimum and maximum loading volumes for the nCounter *SPRINT* Cartridge?

The cartridge has been specified to work with sample volumes between 25-35 µL. After hybridization is complete, ensure the sample volume is between 25-35 µL by adding additional RNase-free water, if needed.

30. If my sample hybridization time is variable, how will that impact raw counts, dynamic range, limit of detection, etc.?

There may be a moderate increase in counts associated with longer hybridization periods. This difference can be normalized during data analysis.

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