



CosMx[®] Best Practices

Volume III

Tips when Generating Single-Cell Spatial Data: Post Run H&E and Antibody Staining

We are excited to share the third installment of tips for successful [CosMx™ SMI](#) single-cell spatial runs at 1000-plex. [Our first installment of tips](#) focused on data by tissue and disease type as well as tissue-specific sample preparation considerations, while our [second installment](#) focused on evaluating tissue block quality, section quality, heterogeneity, and autofluorescence. Here we focus on post-run H&E staining protocols.

Remove Flow Cells

You will need the following materials and reagents for this step: **staining jars, Xylene, razor blade, tape, Kimwipes, 2X SSC.**



Caution

Flow cell removal involves the use of a razor blade and may result in broken glass. The blade and glass present laceration hazards. Follow safety guidelines and wear proper PPE (e.g., hand protection, eye protection, etc.) when performing this step. Dispose of sharps and glass appropriately following your laboratory's safety policy.

Following the post-run clean, remove all flow cells to be imaged from the instrument and place into a staining jar containing Xylene. Leave flow cells in Xylene overnight at room temperature to loosen the flow cell adhesive and allow for easier coverslip removal.

The following day, take proper precautions and carefully remove the flow cell coverslip.

1. Carefully dry the flow cell using a clean Kimwipe being careful to not apply pressure directly to the tissue containing area of the flow cell.
2. Apply a section of tape directly to the top of the flow cell ensuring the entire coverslip is covered but the edges are left free of tape. This will prevent any glass from damaging the tissue should the coverslip crack or break during removal.
3. Use a clean razor blade to lift an edge of the coverslip and then gently slide the razor around the edge of the coverslip to lift the remaining edges (being cautious to avoid injuring your free hand or fingers).

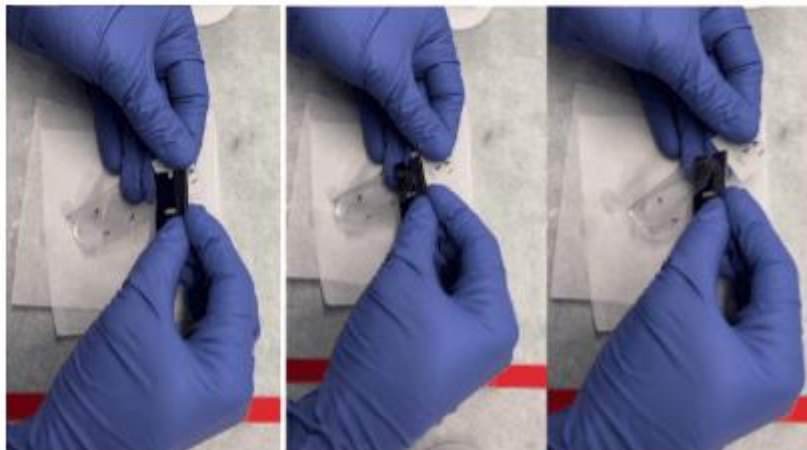


Figure 1: Remove flow cell coverslip

After removing the flow cell coverslip, remove the remaining gelatinous adhesive with the razor and Kimwipe. Wipe off any residual Xylene without allowing tissue to dry, and store in 2X SSC at 4°C until staining.

For the **H&E staining protocol**, [continue to the next section](#). To continue to the **Immunofluorescence (IF) staining protocol** (Antibody staining), [see below](#).

Post Run H&E Staining Protocol

Post Run H&E Staining Preparation

You will need the following materials and reagents for this step: **staining jars**, **SelecTech Hematoxylin 560** (Leica Biosystems [3801570](#)), **SelecTech Defining Solution** (Leica Biosystems [3803590](#)), **SelecTech Blue Buffer 8** (Leica Biosystems [3802915](#)), **SelecTech Alcoholic Eosin Y 515** (Leica Biosystems [3801615](#)), **MM24 Mounting Medium** (VWR [3801120](#)), **Xylene**, **distilled water** (dH₂O).

Dilute SelecTech Blue Buffer 8 **1:20 in distilled water** (dH₂O) before use.

Prepare a total of 16 total staining jars for the H&E staining steps:

Table 1: Prepare Staining Jars

Distilled Water	Label 5 staining jars as dH ₂ O (1-5) and fill each with dH ₂ O.
Hematoxylin	Label one jar as Hematoxylin and fill with Hematoxylin 560.
Defining Solution	Label one jar as Defining solution and fill.
Blue Buffer 8	Label one jar as Blue Buffer and fill with Blue Buffer 8.
Alcoholic Eosin Y	Label one jar as Eosin Y and fill with Alcoholic Eosin Y.



95% ethanol	Label 2 jars as 95% Ethanol (1, 2) and fill with 95% ethanol.
100% ethanol	Label 2 jars as 100% Ethanol (1, 2) and fill with 100% ethanol.
Xylene	Label 3 jars as Xylene (1-3). Fill jars with Xylene and leave in the fume hood until use.

Post Run H&E Staining

Due to the hazardous properties of Xylene, the Xylene steps should be done in the hood following your laboratory guidelines. Hematoxylin, post-hematoxylin water, and Alcoholic Eosin Y are considered dye-containing waste and should be disposed of properly. Defining solution contains alcohol and should be disposed of properly. Dispose of all other chemical waste following your laboratory guidelines.

The following steps are done at **room temperature** unless otherwise noted:

1. Remove slides from 2X SSC and gently submerge slides into the first dH₂O wash for **5 minutes**.
2. Transfer slides to the second jar of dH₂O and wash for **5 minutes**.
3. Remove from dH₂O and gently immerse slides into Hematoxylin and wash for **3 minutes**.
4. Transfer slides to dH₂O (3) and wash for **1 minute**.
5. Transfer slides to Defining solution and **gently move slides up and down 5 times to wash** (about 5 seconds).
6. Transfer slides to dH₂O (4) and wash for **1 minute**.
7. Transfer slides to Blue Buffer 8 and wash for **1 minute and 30 seconds**.
8. Transfer slides to final dH₂O jar (dH₂O (5)) and wash for **1 minute**.
9. Transfer slides to Alcoholic Eosin Y and incubate for **2 minutes**.
10. Transfer slides to the first 95% Ethanol wash (1) and wash for **30 seconds**.
11. Transfer slides to second 95% Ethanol wash (2) and wash for **30 seconds**.
12. Transfer slides to the first 100% Ethanol wash (1) and wash for **30 seconds**.
13. Transfer slides to second 100% Ethanol wash (2) and wash for **30 seconds**.
14. Carry staining jar to **fume hood** and transfer slides to the first Xylene wash. Wash slides for **1 minute**.
15. Transfer slides to Xylene 2 and wash for **1 minute**.
16. Transfer slides to final Xylene wash, and wash for **1 minute**.

Apply Mounting Media and Coverslip

Prior to mounting the coverslip, ensure that the slide is dry. Moisture on the surface of the slide may result in poor mounting.

Wipe away any residual droplets with a Kimwipe being careful not to touch the tissue with the Kimwipe.

1. Place slides on a flat, clean, non-absorbent work surface.
2. Using a **1000 µL pipette**, add **150-200 µL** mounting media to uniformly cover the entire tissue area.
3. Apply the coverslip at an angle on one end of the slide. Slowly lower the coverslip, without introducing bubbles. Allow mounting media to spread and settle.

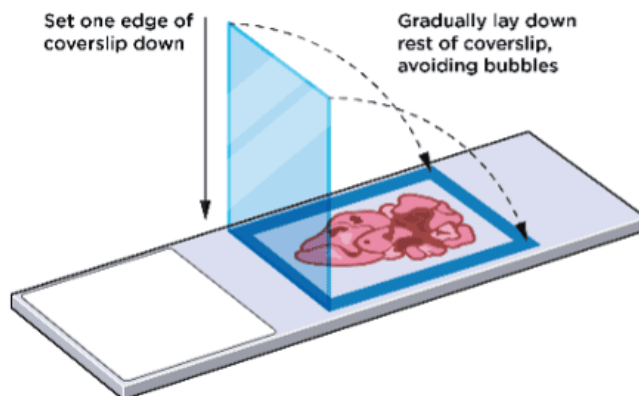


Figure 2: Apply the Coverslip

Samples may be stored temporarily at 4°C and should be imaged within 3 days after mounting.

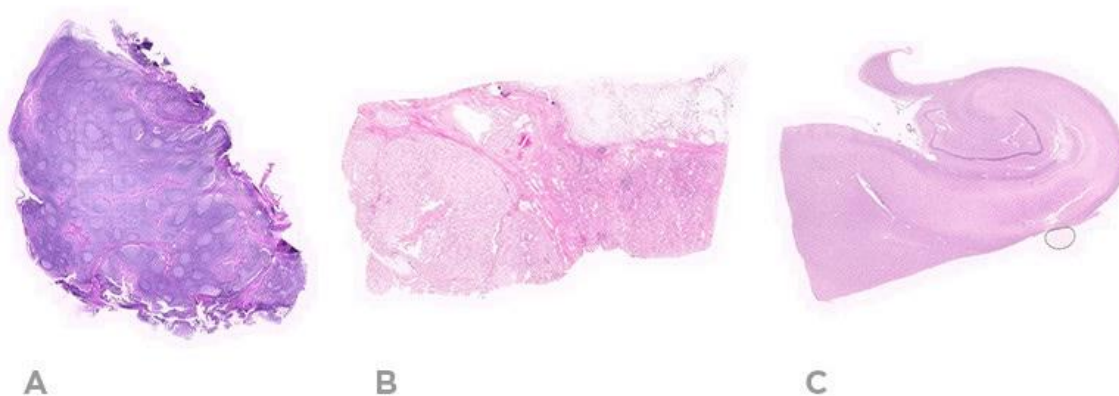


Figure 3: Examples of post-run H&E on (A) Tonsil, (B) Human Renal Cell Carcinoma, and (C) Human Brain Hippocampus.

Some loss of staining efficiency is expected during the post-run H&E. However, the following steps can be used to improve imbalanced H&E images (Figure 4):

1. Non-linear transform of the raw images (e.g., czi format images)
 - Log-transform (using ImageJ/Fuji)
 - Gamma-transform (using ImageJ/Fuji)
2. Rescale the intensity histograms for RGB channels (ImageJ)
3. Rebalance the signal ratio between R and B channels (ImageJ)
 - Enhance Red
 - Suppress Blue
4. Adjust the final appearance of the image.
 - Use Green to finetune white balance.

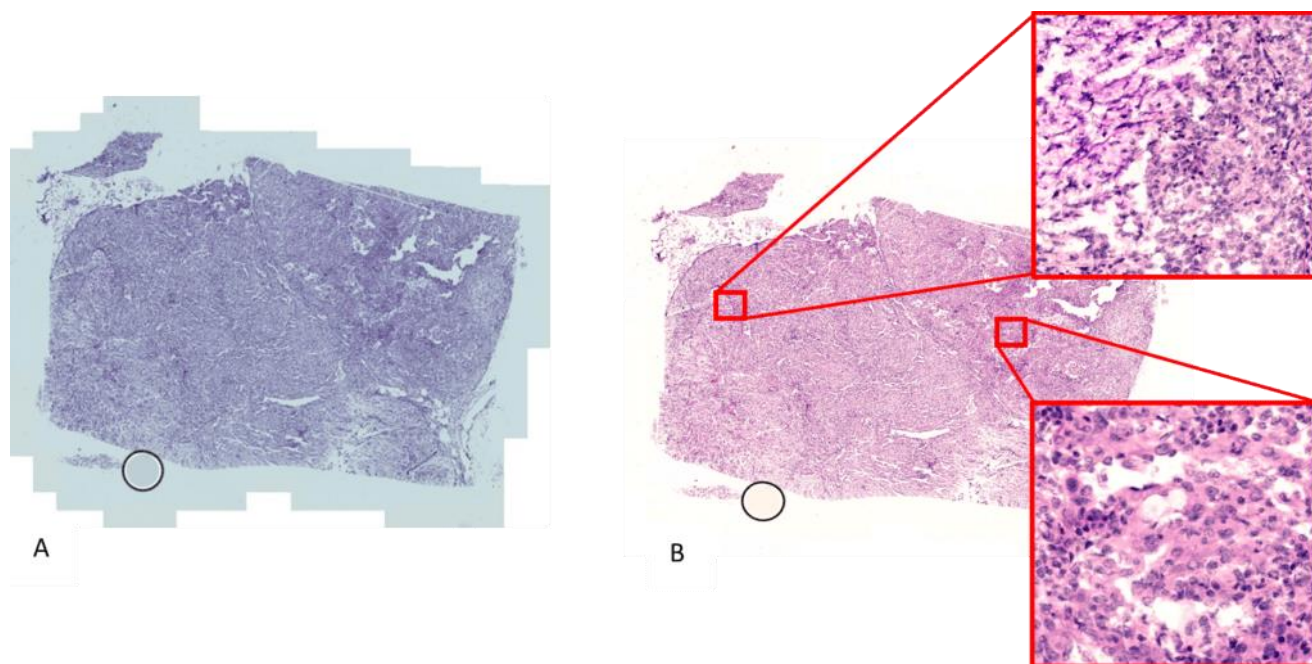


Figure 4: Lung cancer tissue (A) post-run H&E stain with no balance correction, (B) post-run H&E stain following Gamma transform.

Post-run Immunofluorescence Staining Protocol

You will need the following materials and reagents for this step: **staining jars, 2X SSC, compatible fluorescent labeled antibodies, staining tray with cover.**

1. Prepare 5 staining jars of 2X SSC.
2. Following flow cell removal, transfer slides from 2X SSC to a new staining jar of 2X SSC and wash for **5 minutes**.
3. During wash steps, **prepare antibodies according to manufacturer specifications**. Antibody concentrations may need to be adjusted based on empirical testing.
4. Transfer slides to second staining jar of 2X SSC and wash for an additional **5 minutes**.
5. After final SSC wash, gently tap slides on a clean Kimwipe to remove excess SSC and lay flat in a staining tray.
6. Apply **up to 200 μ L** of prepared antibody directly to the tissue. Gently move the tray side to side as needed to ensure that the antibody stain covers the entire tissue.
7. Cover tray and incubate slides for **1 hour at room temperature**.
8. Following incubation, gently tap the slides on a clean Kimwipe to remove excess antibody stain and transfer slides to the next staining jar of 2X SSC. Wash for **5 minutes**.
9. Transfer slides to the next staining jar of 2X SSC and wash for **5 minutes**.
10. Transfer slides to the final staining jar of 2X SSC and wash for **5 minutes**.
11. Coverslip slides and, for best results, continue directly to imaging.

Samples may be stored temporarily at **4°C covered and protected from light** and should be imaged within 3 days.

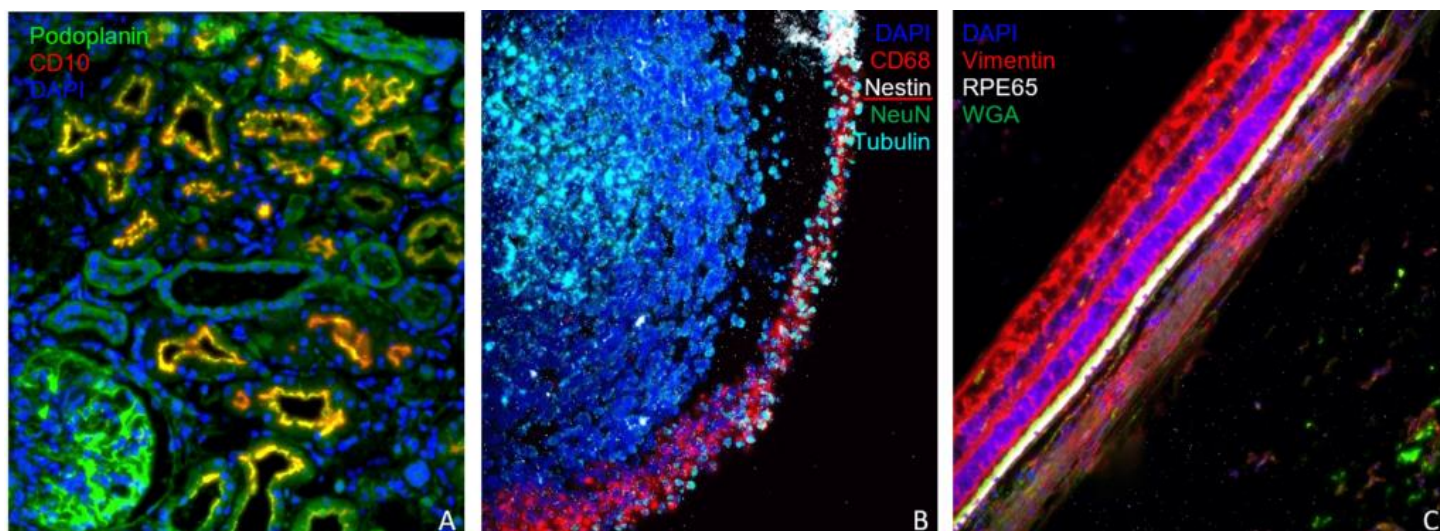


Figure 5: Examples of post-run IF images on FFPE (A) Kidney, (B) Brain Organoid, and (C) Davidson Fixed Retina.

The following antibodies have been tested by Bruker Spatial Biology for post-run IF staining:

NOTE: these antibodies have been tested but not yet validated. The use of any antibody may require empirical testing and optimization. This protocol is optimized for the use of primary conjugated antibodies for post-run IF staining.

Target Antibody	Tested Tissue Type	Antibody Supplier	Antibody Clone
CD20	FFPE kidney FFPE tonsil FFPE skin SCC	Novus	IGEL/773
Laminin	FFPE heart	Novus	NB300-144AF647 (POLYCLONAL)
SMA	FPE colon cancer FFPE ovarian	Abcam	1A4
PMEL17/S100B	FFPE melanoma FFPE skin	Novus	HMB45
beta-amyloid	FF mouse brain	Novus	MOAB-2
GFP	FFPE mouse kidney	Invitrogen	A21311 (POLYCLONAL)
CD10	FFPE kidney	Abcam	EPR22867-118
Podoplanin	FFPE kidney	Agilent	D2-40
NeuN	FFPE brain organoid	Abcam	EPR12763
Tubulin	FFPE brain organoid	Abcam	EP1569Y
Vimentin	Davidson fixed retina	Santa Cruz	E5
PanCK	Universal	Novus	AE-1/AE-3
CD298	Universal	Abcam	EP1845Y
B2M	Universal	Abcam	EP2978Y
CD45	Universal	Novus	2B11+PD7/26
CD3	Universal	Origene	UMAB54
Histone_H3	Universal	Cell Signaling	D1H2
GFAP	Universal	Abcam	EPR19996
CD68	Universal	Abcam	C68/684



Additional antibodies may be used but will require empirical testing. For a list of antibodies and morphology markers tested with the GeoMx® DSP platform, which should be suitable for use with post-run CosMx SMI tissue, visit <https://nanosttring.com/products/geomx-digital-spatial-profiler/geomx-morphology-markers/>.

Special thanks to the Chun lab at the Cumming School of Medicine, University of Calgary; the Ginhoux lab at the Singapore Immunology Network, Agency for Science, Technology, and Research; and the Parfitt Ophthalmology Discovery Research lab at AbbVie for permission to publish the post-run IF images of their tissue samples run through the NanoString Technology Access Program (Figure 5A, B, and C respectively).

Thank you for choosing CosMx. If you have any further questions or need assistance, please don't hesitate to contact our Support team (Support.Spatial@Bruker.com).