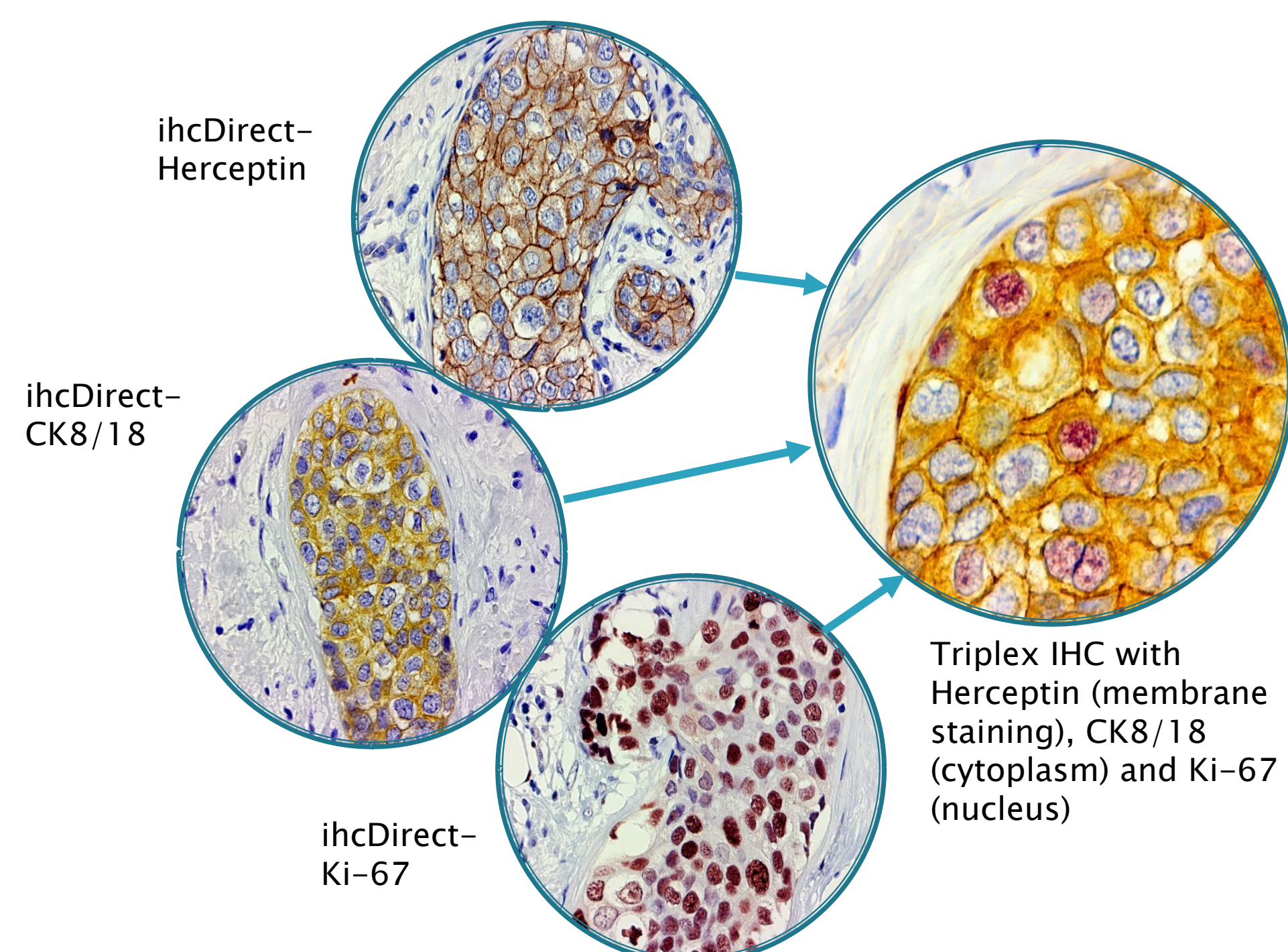


## Background

Multiplex testing can optimize the use of precious specimens, provide better insights into the local microenvironment, reveal a better understanding of co-expression and spatial organization of biomarkers and/or distinguish different cells in blood samples. The Novodiox ihcDirect™ technology, in comparison to conventional immunohistochemistry (IHC), provides a simpler and faster test procedure. Novodiox uses a novel polyHRP-antibody detection system to improve both intraoperative IHC on frozen tissues as well as immunocytochemistry (ICC) on fresh cells. Tests for both processes can be completed in just 10 minutes while demonstrating superior detection sensitivity and maintaining low background across a wide variety of antibodies. Thus, the multiplex approach with Novodiox ihcDirect technology can be a powerful tool to investigate for hidden clues in cell and tissue samples in a rapid fashion. For instance, a triplex IHC/ICC on fresh tissue or cells can be finished within an hour. Recently, this technology has been applied to the analysis of blood samples in liquid biopsy for detecting circulating tumor cells using both colorimetric and fluorescent substrates.



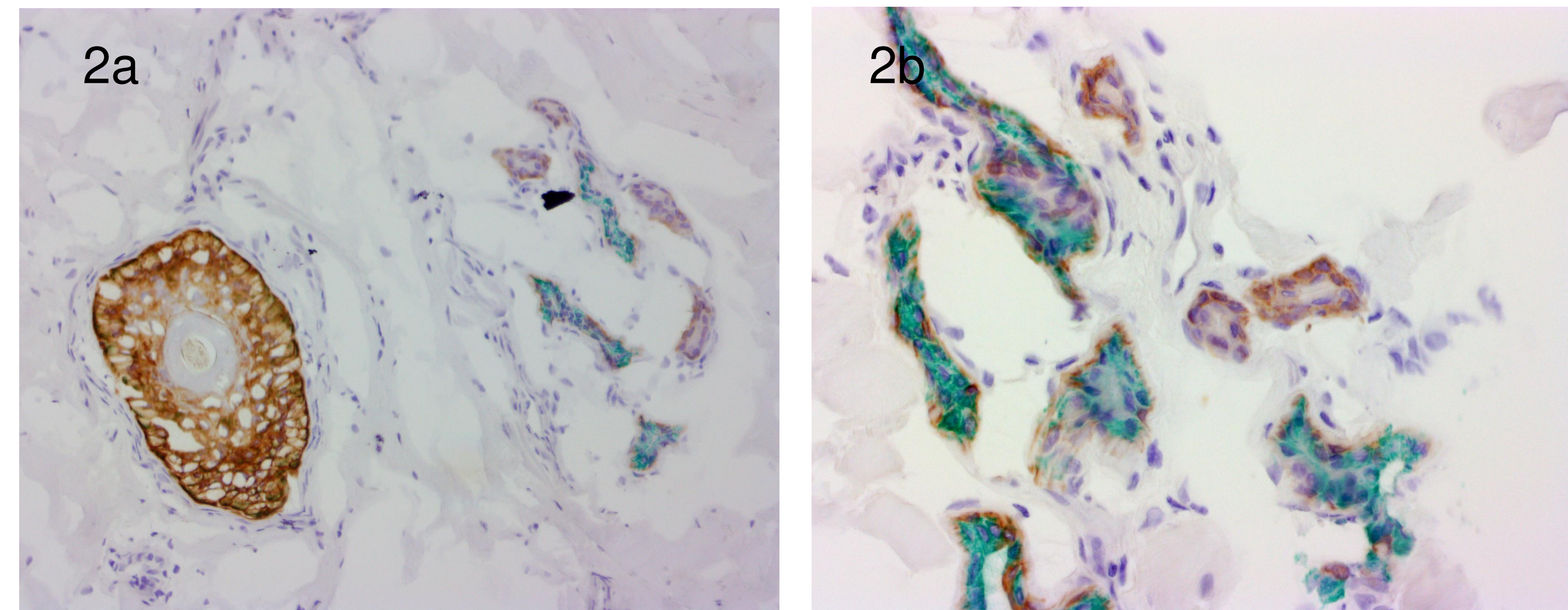
**Figure 1.** Demonstrates a colorimetric IHC multiplex on Breast Cancer formalin-fixed paraffin-embedded (FFPE) tissue. Tissues were stained using ihcDirect Herceptin, ihcDirect CK8/18, and ihcDirect Ki-67. The cancer cell membrane was highlighted with brown color, the cytoplasm with bright yellow, and the nucleus with dark red.

## Results & Discussion

Table 1. A comparison between Novodiox IHC and conventional IHC

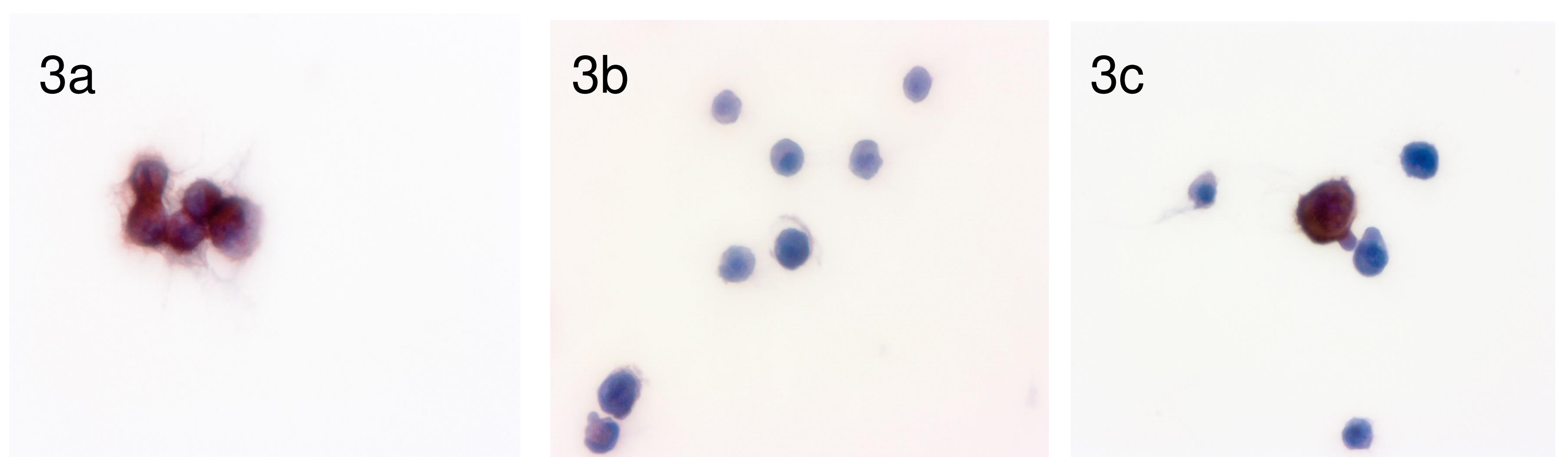
	Novodiox IHC	Regular IHC	Procedure	Novodiox IHC on FF	Novodiox IHC on FFPE	Other IHC on FFPE
<b>Parallel multiplex IHC</b>						
Species limit for primary Ab	No	Yes	Dewax + AR		60	60
Speed	Fast	Slow	Block	1	30	30
			1st IHC	9	45	105
			Stripping or HRP deactivation	10	10	30
<b>Sequential multiplex IHC</b>						
Stripping off primary Ab	No	Yes	Block			30
Diminished signal	No	Yes	2nd IHC	9	45	105
Blocking between IHC	No	Yes	Stripping or HRP deactivation	10	10	30
Speed	Fast	Slow	Block			30
			3rd IHC	9	45	105

Sequential multiplexing of IHC using the Novodiox ihcDirect technology uses a simple 1-step deactivation procedure that requires no antibody stripping. Per Table 1, this method provides a fast and easy way for users to observe multiple bio-markers on frozen or FFPE tissue sections. Thus, this approach is very convenient for researchers to obtain more information from a single sample.



**Figure 2.** Application of a duplex IHC on a Frozen Skin Tissue. The tissue was stained with ihcDirect CK5 (Brown) and ihcDirect CK8/18 (Green).

To more accurately determine margins in surgical treatment of skin cancers, we applied ihcDirect CK5 onto intra-operative frozen sections. CK5 is a cytokeratin that has demonstrated utility when directed at basal cell carcinoma and squamous cell carcinoma and on surgical margins during the skin cancer surgery. However, often the morphology of exocrine gland cells, such as sweat glands, or sebaceous glands can make it difficult to distinguish gland tissue from cancer cells. This situation can make difficult for doctors to provide an accurate diagnosis. As shown in Figure 2, the use of a duplex stain, ihcDirect CK8/18 highlighted the sweat gland. Notably, the overall procedure of using this duplex IHC staining was approximately 30 minutes.



**Figure 3.** Application of Novodiox ihcDirect technology on a fresh cell samples. 3a: Breast Cancer cell stained positive by ihcDirect-Herceptin; 3b: Melanoma cell stained negative by ihcDirect-Herceptin; 3c: Melanoma cell sample spiked with Breast Cancer cell, the breast cancer cell was selectively stained by ihcDirect-Herceptin. All ihcDirect-antibodies were developed by Novodiox Inc. The fresh breast cancer cell was SK-Br3, and the melanoma cell was SK-Mel. Before the ICC staining, the cell culture was added onto a clean slide and dried in a 37°C incubator, then fixed for 30 seconds in a formalin fixative.

A similar strategy can also be applied to fresh cell samples obtained from liquid biopsies. Under these conditions the Novodiox ihcDirect polyHRP technology permitted rapid visualization of different cells specific to the conjugated antibody.

## Conclusion

Rapid multiplex testing of specimens using Novodiox ihcDirect for IHC/ICC is achievable and shows great promise to aid researchers and medical professionals obtain timely and useful information. This technology facilitates fast and accurate diagnosis for frozen tissues, FFPE and circulating tumor cells applied to basic research and medicine.