Development and validation of an assay for the characterization of SSTR2 expression on CTCs from liquid biopsies

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Memorial Sloan Kettering Cancer Center

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BACKGROUND

Expression of Somatostatin Receptor subtype 2 (SSTR2) is observed in multiple cancers where its role as a prognostic or predictive marker is cancer-type dependent. There has been renewed interest in SSTR2 and the critical role it plays as a target for radiotherapeutics (peptide receptor radionuclide therapy – PRRT) in patients with neuroendocrine tumors (NETs). In this study, we describe the development and validation of a novel assay to analyze circulating tumor cells (CTCs) for expression of SSTR2 using the RareCyte platform.

METHODS

After AccuCyte® processing, an unbiased density-based method for collecting and transferring nucleated cells from whole blood to slides, we stained for SSTR2, CD45, and CK/EpCAM to identify CTCs (CK+, EpCAM+, CD45-), and quantitate SSTR2 expression. Three SSTR2 clones were tested to determine which one provided the optimal signal to background ratio (S:B). The preferred clone was titrated to establish the optimal concentration for the assay. Synaptophysin (SYP) testing had previously been optimized on this platform. Slides were imaged with CyteFinder®, an automated multiparameter immunofluorescent (IF) microscopy system that applies machine learning algorithms for rare cell identification. Spike-in and clinical samples were used for assay validation. Single-cell SSTR2 mean fluorescence intensities (MFI) were measured to determine expression levels. Colorectal cancer with neuroendocrine features and Merkel cell cancer (neuroendocrine skin tumor) patient samples were evaluated for CTC enumeration and SSTR2 and SYP expression.

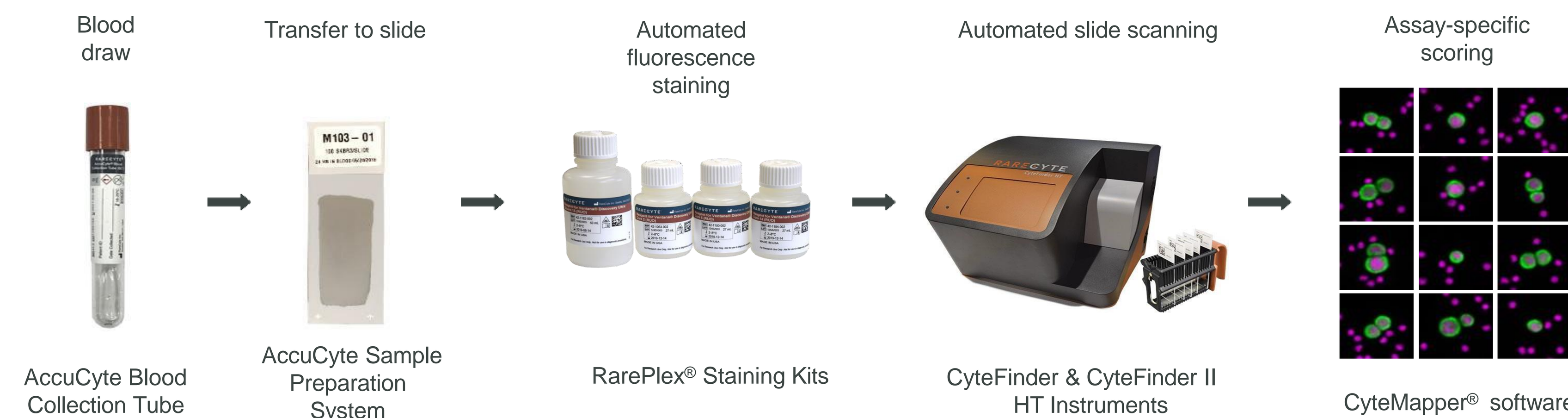

Figure 1. RareCyte platform workflow. Blood is collected into AccuCyte Blood Collection Tubes (BCTs). Nucleated blood cells are processed to slides using the AccuCyte Sample Preparation System. Slides are stained with assay-specific RareCyte staining kits. Slides are scanned using the CyteFinder Instrument and images are analyzed using CyteMapper software and analysis tools. Cells are analyzed by a trained reviewer, and cell biomarker status is determined with a fluorescence intensity threshold.

RESULTS

The assay's performance metrics for SSTR2 expression were: 93% accuracy, 85% sensitivity, and 100% specificity. A small cohort of patient blood samples were tested using the assay. The percentage of SSTR2-positive CTCs ranged from 0-60%, reflective of tumor heterogeneity.

Biomarker	Antibody Clone	Median Positive MFI	Median Negative MFI	S:B
SSTR2	UMB1	1923.3	213.9	9.0
	1C3	1831.1	581.9	3.1
	402038	1337.0	53.5	25.0

Table 1. Median MFI of positive and negative spike-in cells and signal to background (S:B) of each antibody clone tested for SSTR2 detection. Copper border indicates the clone with the optimal S:B.

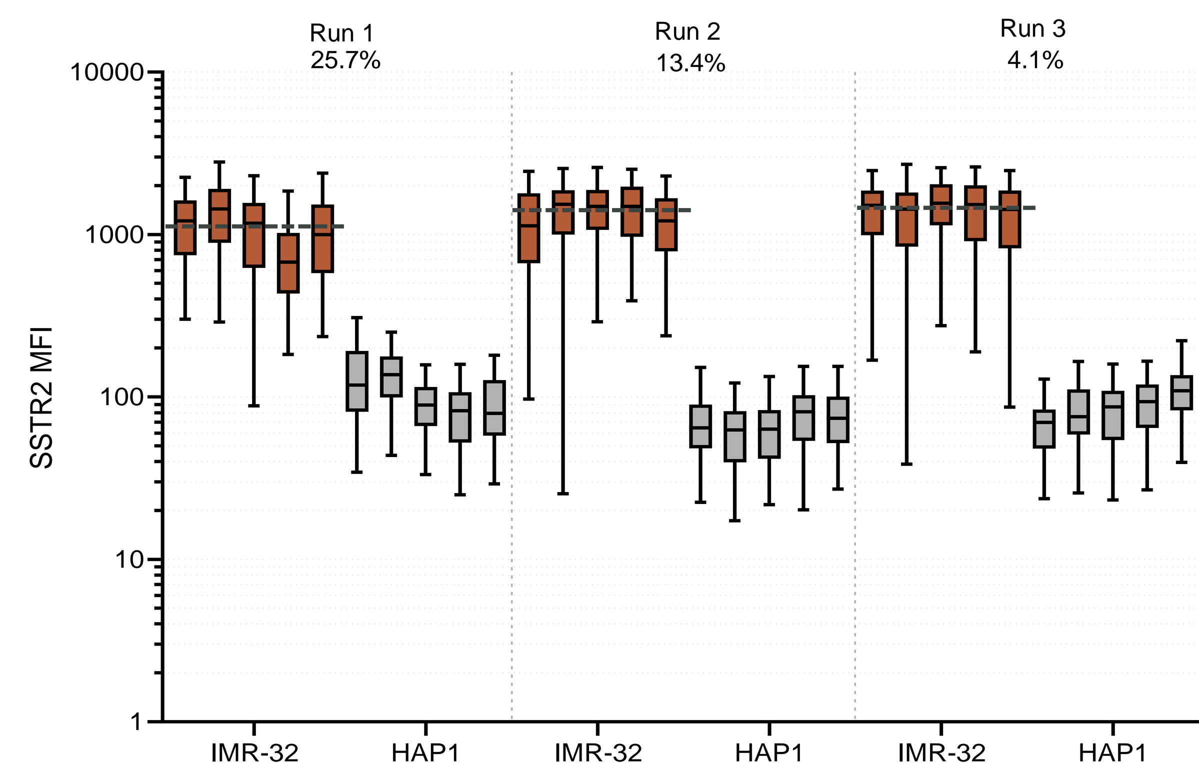

Figure 2. Performance characterization of SSTR2 Assay
Distribution of SSTR2 MFI on IMR-32 (copper, positive) and HAP1 (light grey, negative) spike-in cells for each slide on three replicate runs. Each group represents 1 slide. Repeatability is the CV of the median SSTR2 MFI (dashed copper line) across replicate slides within a single run and is indicated above each group. Average CV of 3 runs is 14.4%. Whiskers span 5 – 95 percentiles for all graphs.

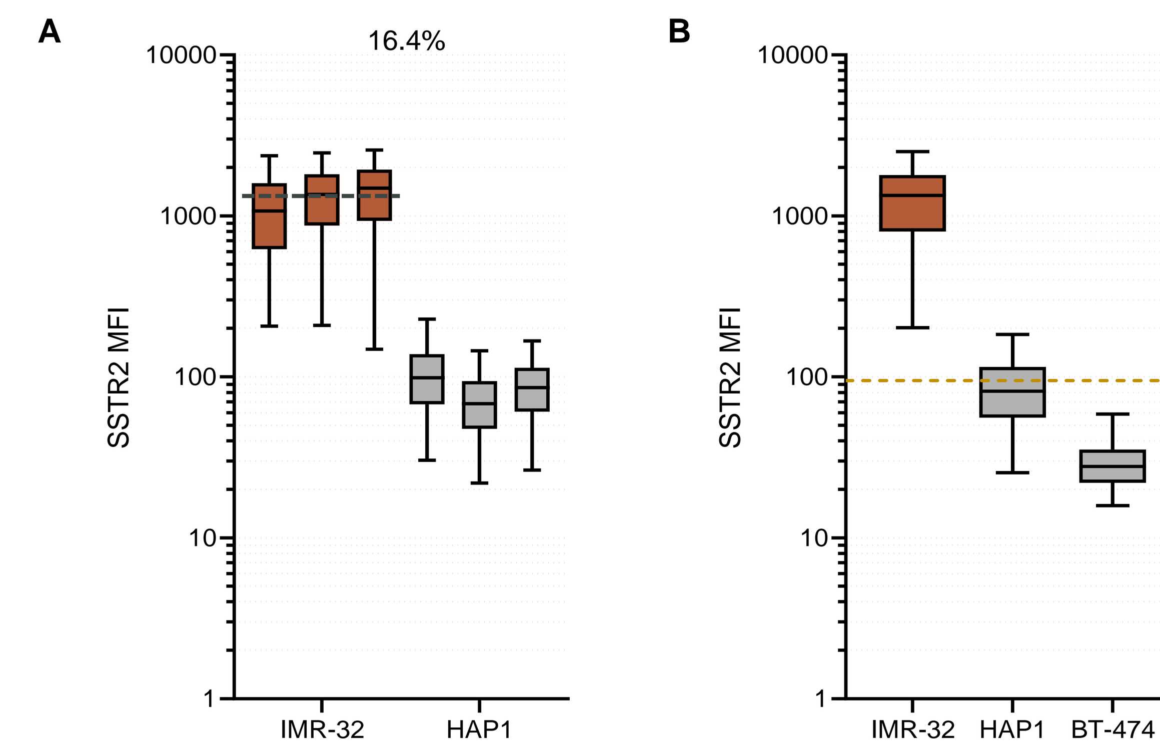

Figure 3. Performance characterization of SSTR2 Assay
(A) Distribution of SSTR2 MFI on IMR-32 (copper, positive) and HAP1 (light grey, negative) spike-in cells for three replicate runs. Each group represents 5 slides. Inter-stainer run precision is the CV around the median SSTR2 MFI (Dark grey dashed line) for 3 independent stainer runs and is indicated on graph. (B) Distribution of SSTR2 MFI on IMR-32 (copper, positive), HAP1 (light grey, negative), and BT-474 (light grey, negative) spike-in cells for three replicate runs. Each group represents 15 slides, except the BT-474 group which represents 2 slides. Preliminary positivity threshold (95) indicated by gold dashed line. Whiskers span 5 – 95 percentiles for all graphs.

mCTC	SSTR2	Cancer type
IMR-32	+	Neuroblastoma
HAP1	-	Chronic myelogenous leukemia
BT-474	-	Mammary gland epithelial

Table 2. List of cell lines used as model CTCs and their expected SSTR2 expression.

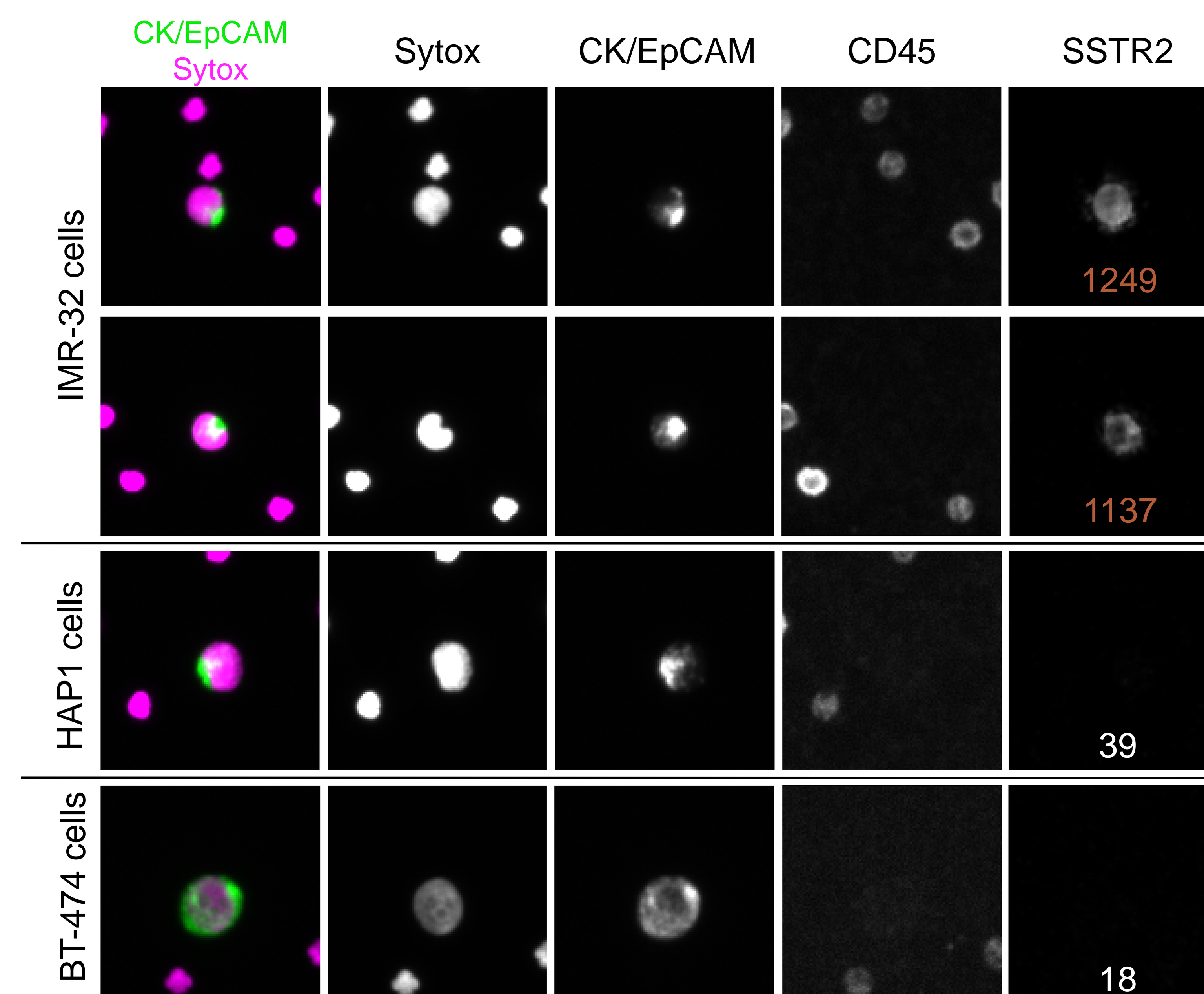

Figure 4. Representative images of single biomarker SSTR2 assay staining
Representative IMR-32, HAP1, and BT-474 spike-in cells stained with SSTR2 assay. Mean fluorescence intensity (MFI) shown for each CTC in copper (positive) or white (negative) below each cell. Cells are representative of the median MFI signal observed.

Patient	CTC Count	% + for SSTR2	% + for SYP	Cancer type
CRC1	362	56	98	Colorectal
MCC1	151	49	68	Merkel Cell
MCC2	234	24	43	Merkel Cell
MCC3	8	0	13	Merkel Cell
MCC4	15	13	73	Merkel Cell

Table 3. Patient Data of SSTR2 and SYP Assay
CTC count, SSTR2 % positivity, SYP % Positivity, and cancer type of each patient sample tested.

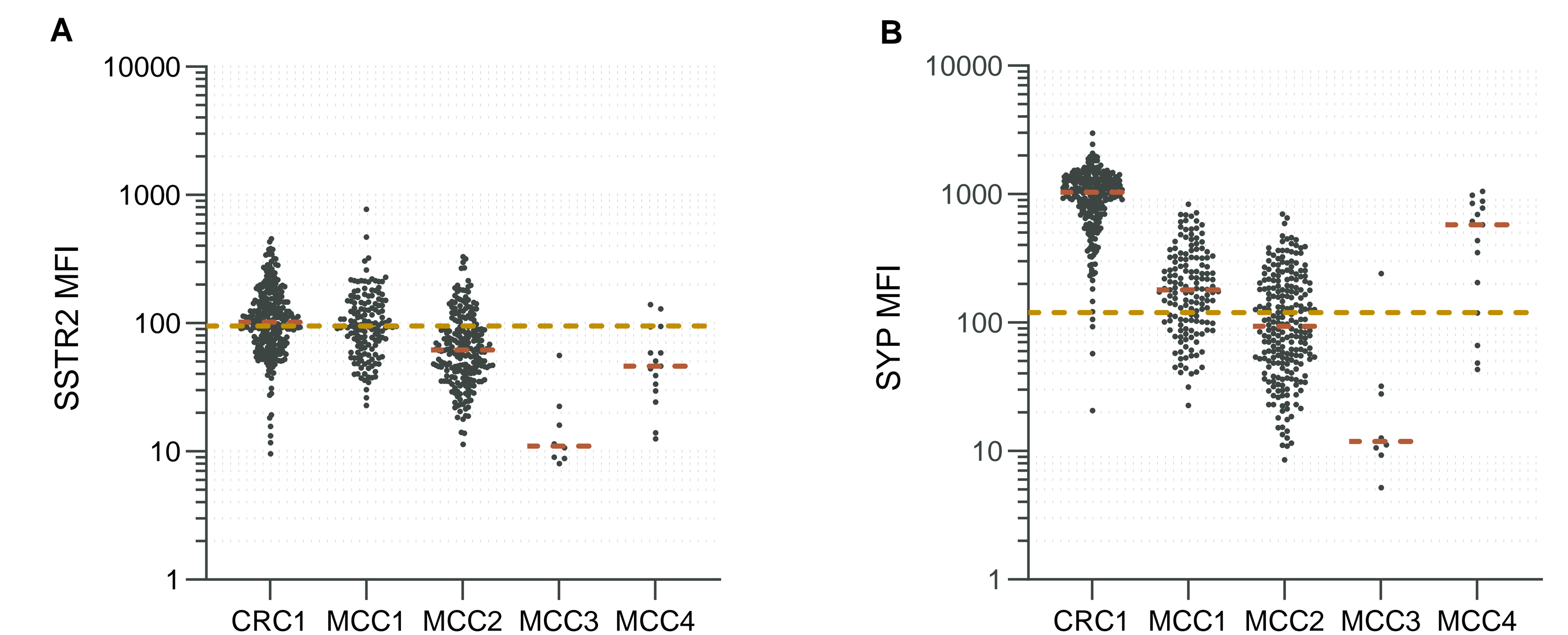

Figure 5. Validation of SSTR2/SYP Assay with Clinical Samples
(A) SSTR2 MFI of CTCs from each patient. Median SSTR2 MFI for each patient indicated by copper dashed lines. SSTR2 preliminary positivity threshold (95) indicated by gold dashed line. (B) Synaptophysin MFI of CTCs from each patient. Median SYP MFI for each patient indicated by copper dashed lines. SYP preliminary positivity threshold (120) indicated by gold dashed line.

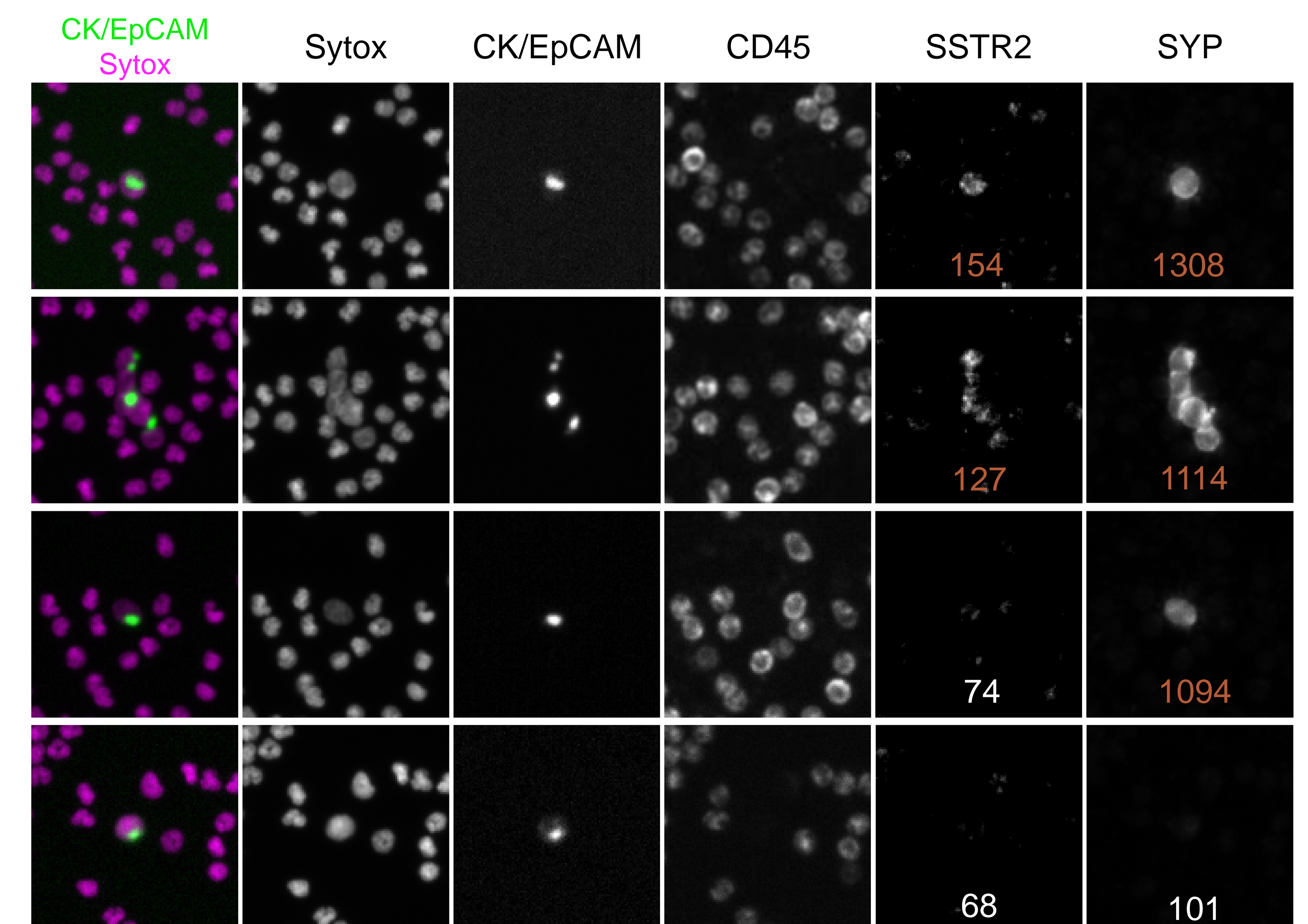

Figure 6. Representative images of dual biomarker SSTR2/SYP assay staining on patient samples.
Representative CTC images from clinical slides stained with the SSTR2/SYP assay. Mean fluorescence intensity (MFI) shown for each CTC in copper (positive) or white (negative) below each cell.

CONCLUSIONS

- Successful development of a novel assay to quantify SSTR2 expression in CTCs with high accuracy, sensitivity, and specificity
- Immunofluorescent microscopy allows for high-resolution imaging of cell morphology and biomarker localization
- Assay validated with small cohort of clinical samples from multiple cancer types
- SSTR2 and SYP expression within and between patient samples exemplifies tumor heterogeneity