

Validation of multiplex immunofluorescence panels using multispectral microscopy for immune-profiling of formalin-fixed and paraffin-embedded human tumor tissues

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Supplementary Table 1. Correlation between chromogenic immunohistochemistry (IHC) and multiplex immunofluorescence (mIF) groups.

Marker	*mIF Batch 1 vs IHC	*mIF Batch 2 vs IHC	*mIF Batch 3 vs IHC
Panel 1			
AE1/AE3	r = 0.273 P = 0.417	r = 0.527 P = 0.096	r = 0.200 P = 0.555
PD-L1	r = 0.618 P = 0.043	r = 0.691 P = 0.019	r = 0.627 P = 0.039
CD4	r = 0.709 P = 0.015	r = 0.764 P = 0.006	r = 0.855 P = 0.001
CD8	r = 0.764 P = 0.006	r = 0.555 P = 0.077	r = 0.773 P = 0.005
CD3	r = 0.791 P = 0.004	r = 0.782 P = 0.005	r = 0.909 P = 0.0001
CD68	r = 0.173 P = 0.612	r = 0.382 P = 0.247	r = 0.873 P = 0.001
Panel 2			
AE1/AE3	r = 0.427 P = 0.190	r = 0.655 P = 0.029	r = 0.382 P = 0.247
Granzyme B	r = 0.173 P = 0.612	r = 0.018 P = 0.958	r = 0.400 P = 0.223
CD57	r = 0.764 P = 0.006	r = 0.527 P = 0.096	r = 0.746 P = 0.009
CD45RO	r = 0.300 P = 0.370	r = 0.346 P = 0.298	r = 0.246 P = 0.467
PD-1	r = 0.846 P = 0.001	r = 0.918 P < 0.0001	r = 0.836 P = 0.001
FOXP3	r = 0.434 P = 0.183	r = 0.410 P = 0.210	r = 0.229 P = 0.499

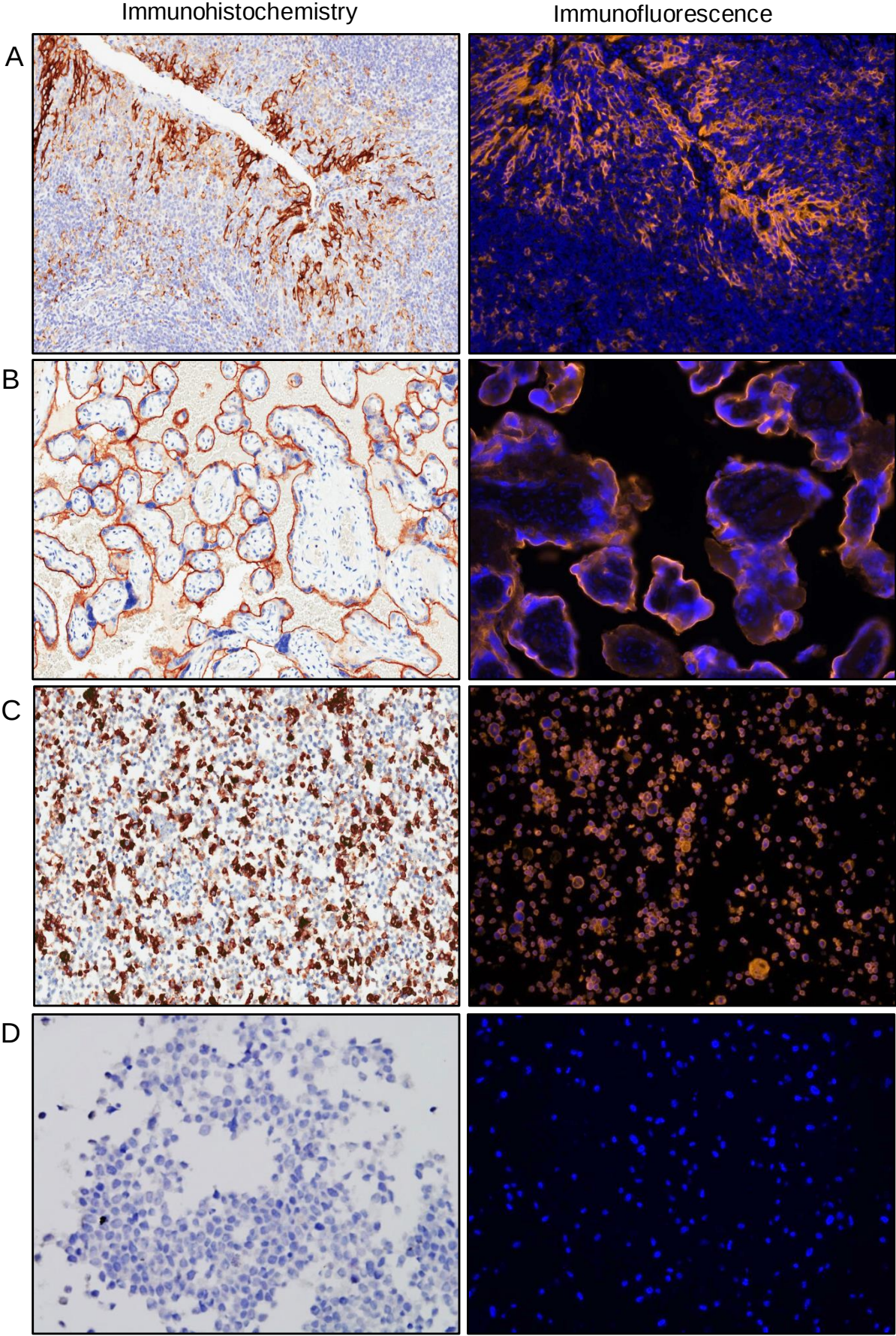
Note: *Spearman correlation.

Supplementary Table 2. Correlation between multiplex immunofluorescence staining batches.

Marker	*Batch 1 vs Batch 2	*Batch 1 vs Batch 3	*Batch 2 vs Batch 3
Panel 1			
AE1/AE3	$r = 0.836$ $P = 0.001$	$r = 0.800$ $P = 0.003$	$r = 0.864$ $P = 0.001$
PD-L1	$r = 0.773$ $P = 0.005$	$r = 0.718$ $P = 0.013$	$r = 0.800$ $P = 0.003$
CD4	$r = 0.918$ $P < 0.0001$	$r = 0.827$ $P = 0.002$	$r = 0.746$ $P = 0.009$
CD8	$r = 0.200$ $P = 0.555$	$r = 0.709$ $P = 0.015$	$r = 0.627$ $P = 0.039$
CD3	$r = 0.918$ $P = 0.0001$	$r = 0.827$ $P = 0.002$	$r = 0.746$ $P = 0.009$
CD68	$r = 0.700$ $P = 0.017$	$r = 0.318$ $P = 0.340$	$r = 0.336$ $P = 0.312$
Panel 2			
AE1/AE3	$r = 0.891$ $P = 0.0002$	$r = 0.655$ $P = 0.029$	$r = 0.600$ $P = 0.051$
CD57	$r = 0.736$ $P = 0.010$	$r = 0.927$ $P < 0.0001$	$r = 0.836$ $P = 0.001$
Granzyme B	$r = 0.482$ $P = 0.133$	$r = 0.482$ $P = 0.133$	$r = 0.555$ $P = 0.077$
CD45RO	$r = 0.727$ $P = 0.011$	$r = 0.882$ $P = 0.0003$	$r = 0.755$ $P = 0.007$
PD-1	$r = 0.918$ $P < 0.0001$	$r = 0.936$ $P < 0.0001$	$r = 0.927$ $P < 0.0001$
FOXP3	$r = 0.133$ $P = 0.696$	$r = 0.575$ $P = 0.064$	$r = 0.430$ $P = 0.187$

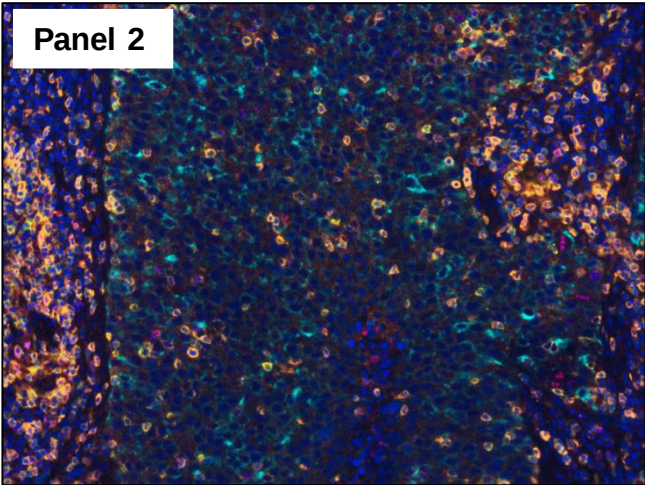
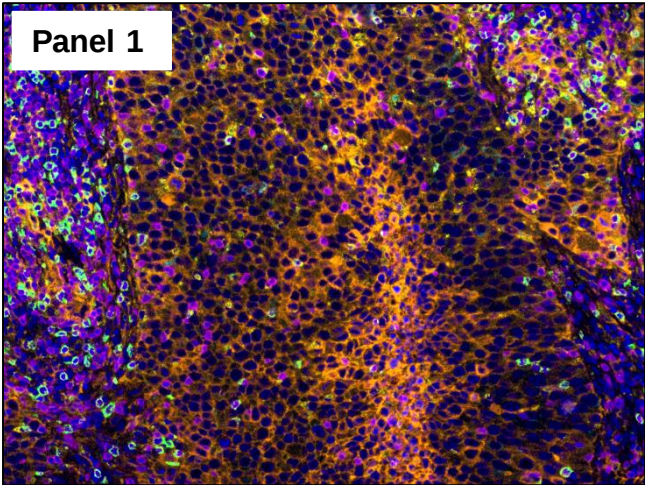
Note: *Spearman correlation.

Supplementary Figure 1. Microphotographs of representative examples of validation from chromogenic IHC and uniplex IF PD-L1 expression. PD-L1 shown membranous expression in (a) epithelial tonsil crypts, (b) placenta syncytiotrophoblasts, and (c) Hodgkin disease–derived cell line (HDLM2), and negative PD-L1 expression in (d) prostate cancer cell line (PC3). ×200 magnification.

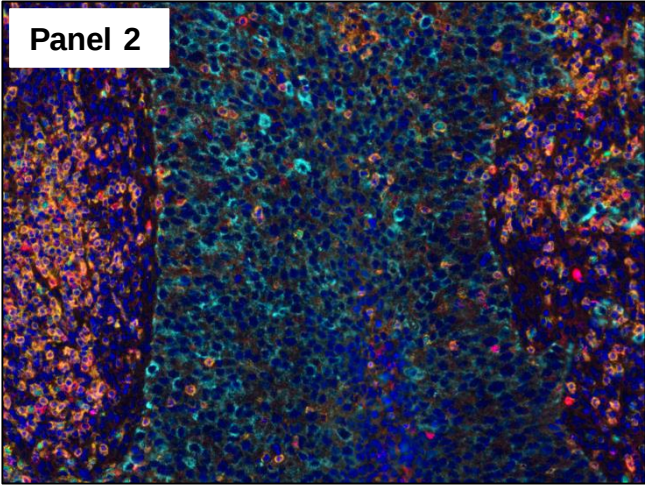
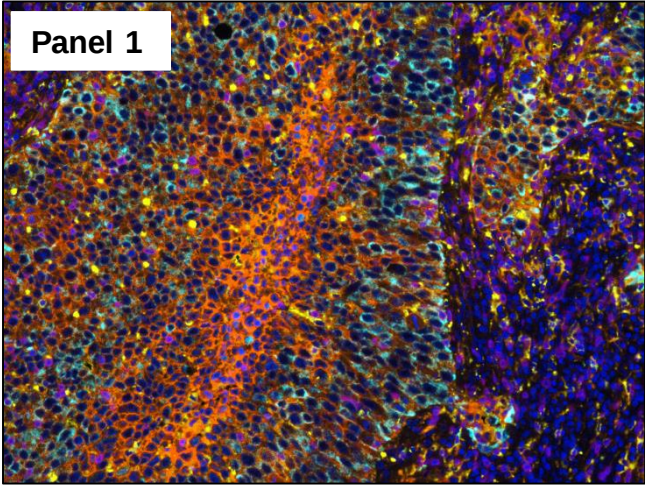


Supplementary Figure 2. Microphotographs of representative examples of staining variation between staining times in panels 1 and 2. Multiplex IF markers in a case of non-small cell lung carcinoma. ×200 magnification.

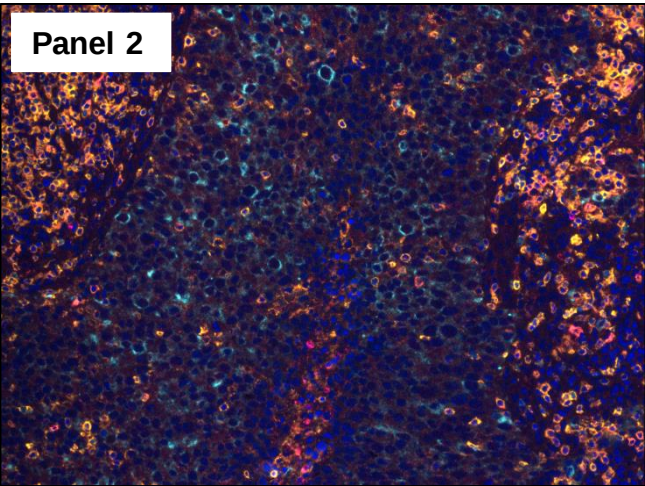
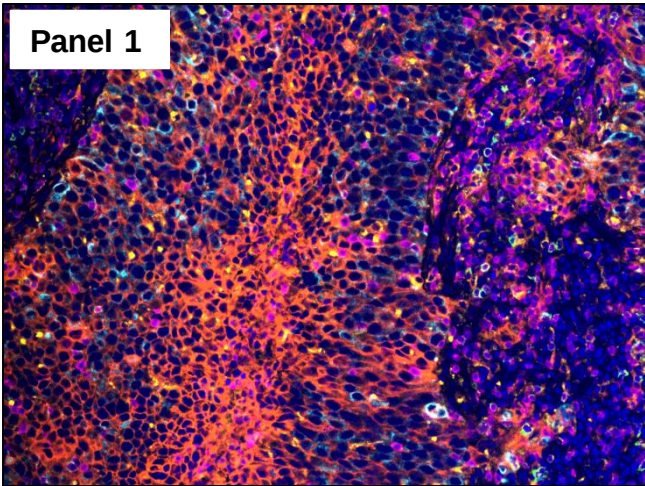
First week of staining



Second week of staining

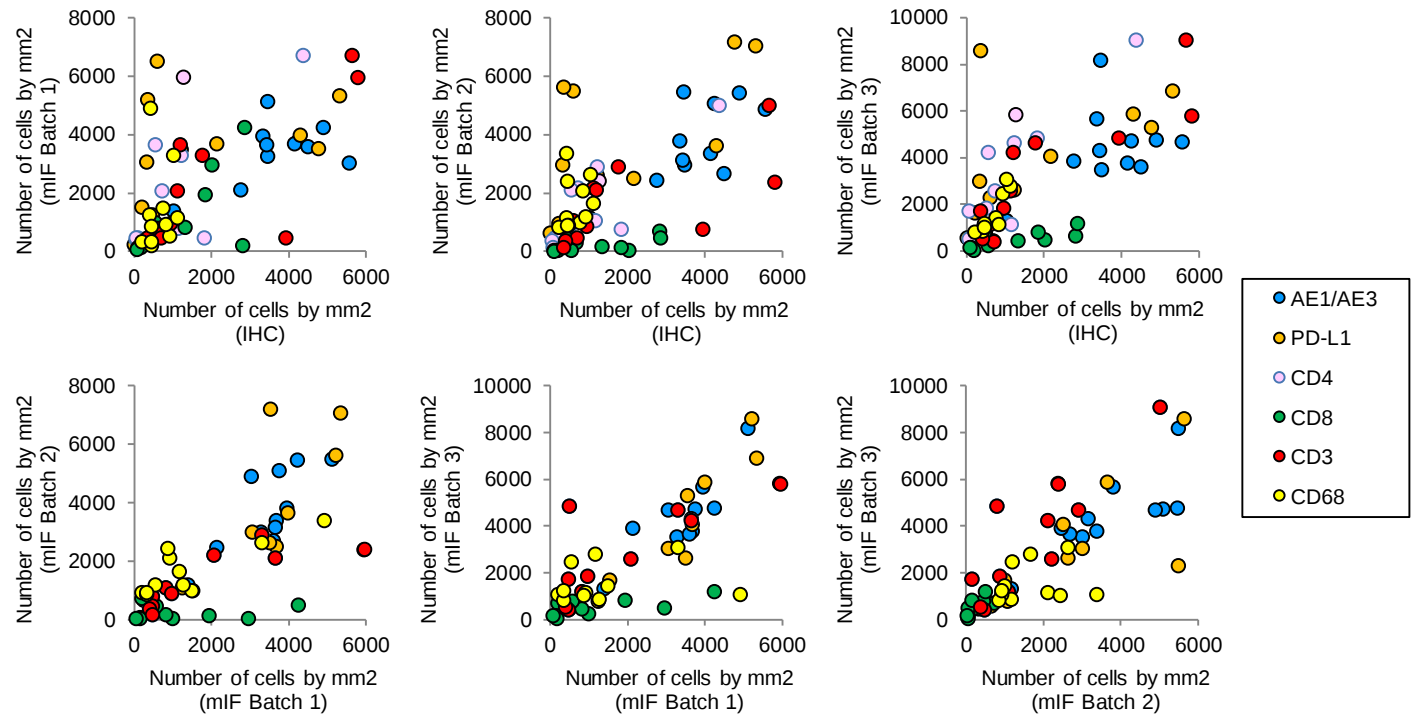


Third week of staining



Supplementary Figure 3. Scatter plots compare the correlation between IHC densities and multiplex IF markers in panels 1 and 2 in the different batches.

Panel 1



Panel 2

