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Online Supplement for:

Semi-automated Digital Image Analysis of Pick's Disease and TDP-3 Proteinopathy

in

Journal of Histochemistry & Cytochemistry

By

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Supplemental Figures: 3 (Supplementary Figure 1-3)

Supplemental Tables: 5 (Supplementary Tables 1-5)

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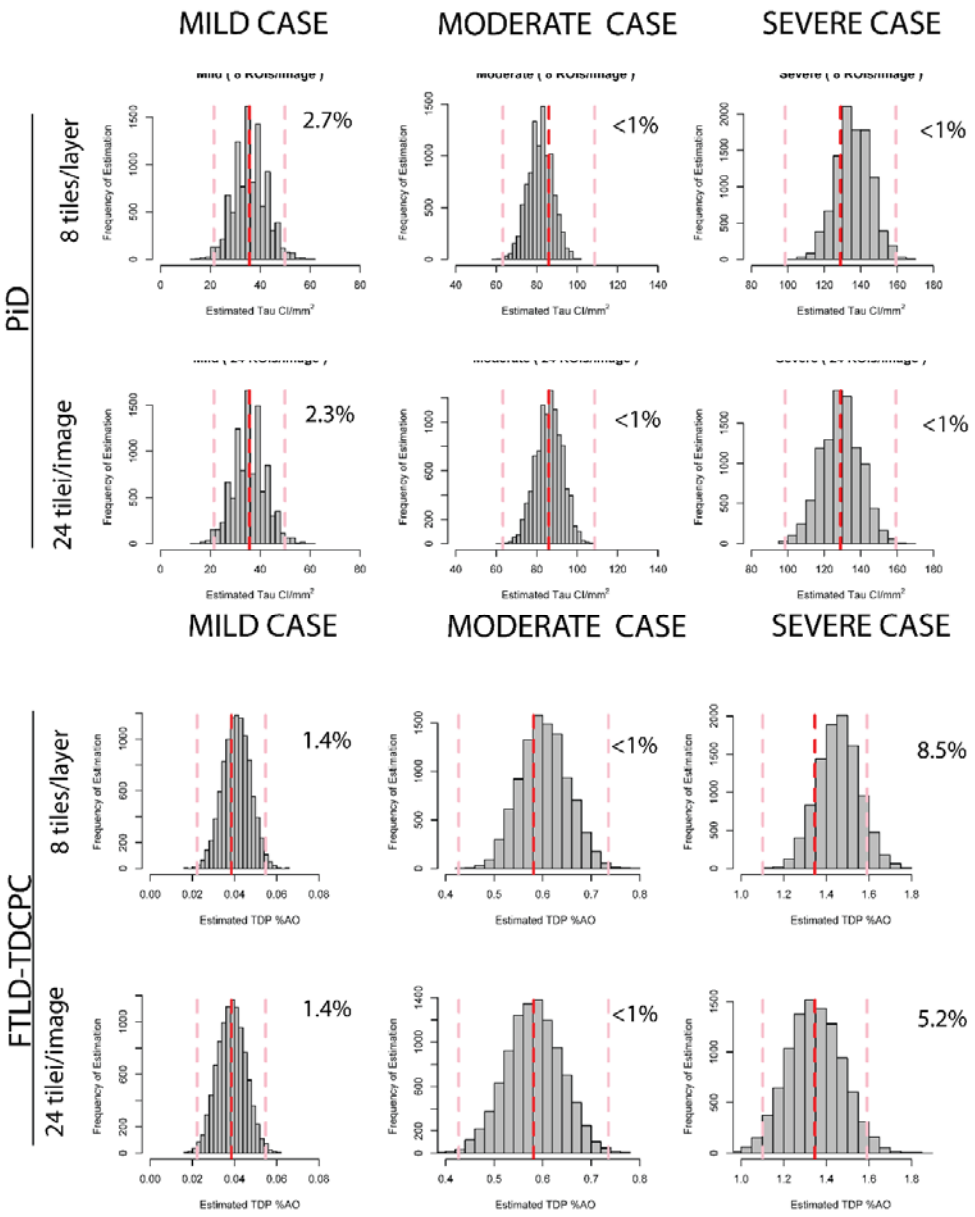

Supplementary Figure 1. Permutation analysis to determine optimal random sampling size. Historgrams depict the frequency of estimations for mean Tau CI/mm^2 (PiD) and TDP %AO (FTLD-TDPC) obtained from 10,000 permutations for either 8 random tiles per cortical layer (24 total tiles) or 24 random tiles sampled independent of cortical layer in a case with mild, moderate and severe burden of pathology. Red dashed line represents the "true" mean value obtained from the full ROI and pink dashed lines represent the limits of 99% CI. Percentages represent the percentage of

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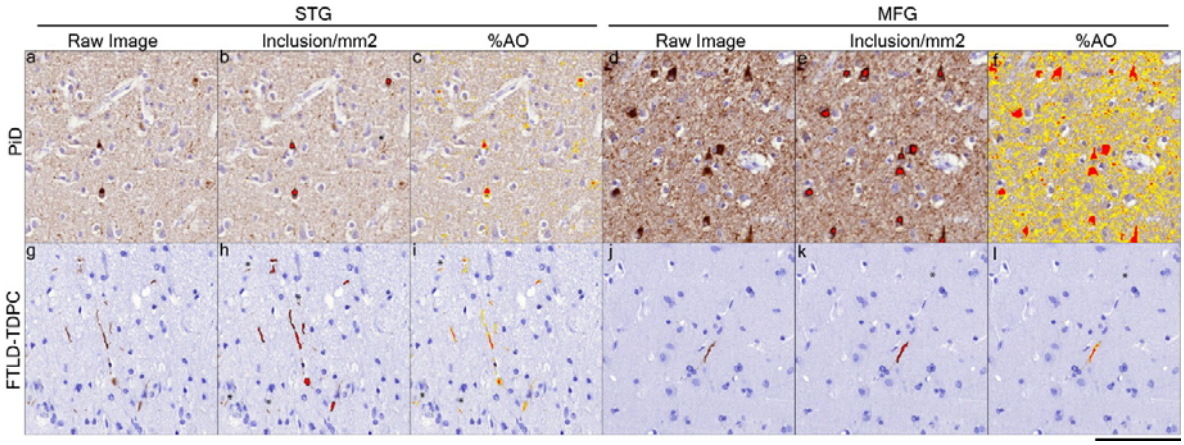
permutation estimations falling outside of the 99% CI. We defined an optimum number of random tiles for sampling is defined as ≤10% of permutation estimates outside of the 99% CI of the true signal; Thus, 24 tiles/image sampling scheme was chosen for analyses.



Supplementary Figure 2. Inclusion/area and %Area occupied digital image analysis for PiD and FTLD-TDPC. Photomicrographs depict representative images from STG (a-c, g-i) and MFG (d-f, j-l) from PiD (a-f) and FTLD-TDPC (g-l) cases using automated digital image analysis algorithms to detect (red-overlay) tau-positive PBs (b,e) and TDP-43 DNs (h,k) and total %AO with positive reactivity (red, orange, yellow) for tau (c,f) and TDP-43 (i,l). PiD had a lower burden of pathology in STG compared with MFG and conversely, FTLD-TDPC had a lower inclusion burden in MFG compared with STG. PiD had a large number of additional small dystrophic neurites included in %AO (c,f) not quantified with PB detection algorithms (b,e) while FTLD-TDPC had minimal additional small DNs in %AO not quantified in inclusion detection algorithms (h, k; asterisks). Scale bar= 100µm.

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Supplementary Table 1. Accuracy of step-wise variation in digital image analysis algorithm variables to determine optimal algorithm in training and test set images.

Path Group	Parameters	R^2 Training Set	R^2 Test Set	R^2 Batch #2	Path Group	Parameters	R^2 Training Set	R^2 Test Set	R^2 Batch #2
PiD	BR=11.0 CT=0.470 OD=0.217	0.421	-	-	**TDP**	BR=1.0 CT=0.196 OD=0.147	0.66	-	-
	BR=11.5 CT=0.470 OD=0.217	0.551	-	-		BR=0.2 CT=0.196 OD= 0.147	0.68	-	-
	BR=12.0 CT=0.470 OD=0.217	0.667	-	-		BR=0.4 CT=0.196 OD= 0.147	0.68	-	-
	BR=11.0 CT=0.475 OD=0.217	0.586	-	-		BR=1.7 CT=0.196 OD= 0.147	0.63	-	-
	BR=11.5 CT=0.475 OD=0.217	0.687	-	-		BR=0.4 CT=0.196 OD=0.157	0.64	-	-
	BR=12.0 CT=0.475 OD=0.217	0.735	-	-		BR=0.4 CT=0.196 OD=0.137	0.69	-	-
	BR=11.0 CT=0.480 OD=0.217	0.707	-	-		BR=0.4 CT=0.196 OD 0.127	0.69	-	-
	BR=11.5 CT=0.480 OD=0.217	0.739	-	-		**BR=0.4 CT=0.196 OD=0.127**	**0.70**	**0.90**	**0.76**

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	BR=12.0 CT=0.485 OD=0.217	0.723	-	-	
	BR=11.5 CT=0.480 OD=0.175	0.783	-	-	
	BR=11.5 CT=0.480 OD=0.155	**0.794**	**0.90**	**0.83**	

BR= blur radius (0-30 scale), CT= contrast threshold (0-50 scale), OD=minimal stain optical density (0-2.4 scale). Optimal algorithms were applied to preliminary manual counts in test set and slides stained in separate IHC batch.

Supplementary Table 2. Optimal Detection Algorithms

Parameter	Tau Inclusions/mm2	Tau %AO	TDP Inclusions/mm2	TDP %AO
Object (Pathology) Stain (RGB) OD	0.838,1.628,1.260	0.838,1.628,1.260	0.296,0.433,0.493	0.296,0.433,0.493
Counter Stain (RGB) OD	0.197,0.236,0.144	0.197,0.236,0.144	0.313,0.313,0.097	0.313,0.313,0.097
Minimum Tissue OD	0.032	0.032	0.005	0.005
Tissue Edge	0	0	0	0

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Thickness				
Object BR	11.5	0	0.4	0
Object Contrast Radius	5	NA	4.493	NA
Object CT	0.48	NA	0.196	NA
Object Stain Min. OD	0.155	NA	0.117	NA
Stain Min. OD (strong, moderate, weak)	NA	0.291,0.500,0.744	NA	0.182,0.288,0.485
Object Size (min, max μm^2)	25.600,630.000	NA	10.000,100000.000	NA
Object connect length (µm)	5	NA	5	NA
Object fill holes	False	NA	True	NA

OD= optical density, BR= Blur radius, CT= Contrast Threshold

Supplementary Table 3. Raw data of final outputs for Tau CI/mm^2 and TDP %AO from ROIs independently segmented by separate investigators.

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Case	Diagnosis	Region	Inclusion/mm^2 Investigator #1	Inclusion/mm^2 Investigator #2	Inclusion/mm^2 Investigator #3
1	PiD	MFG	52.05	59.78	61.23118333
		STG	30.52	43.91	50.69
		ACG	63.01	34.25	30.13
2	PiD	MFG	96.27	92.36	106.45
		STG	35.61	42.46	6.85
		ACG	0.00	0.00	2.76
3	PiD	MFG	64.38	47.82	86.29
		STG	78.83	70.57	92.82
		ACG	16.44	24.47	76.71
4	PiD	MFG	73.03	63.69	80.36
		STG	233.49	147.85	157.41
		ACG	28.76	27.43	12.34
5	PiD	MFG	266.89	184.10	268.88
		STG	152.04	163.00	168.48
		ACG	1.37	36.28	20.57
6	PiD	MFG	52.71	40.73	84.32
		STG	85.27	86.41	102.73
		ACG	45.78	25.01	54.97
7	PiD	MFG	134.89	125.51	134.43
		STG	97.48	108.63	75.48
		ACG	58.97	60.27	57.60
8	PiD	MFG	60.31	72.60	78.61
		STG	93.40	133.28	119.87
		ACG	153.28	150.57	137.74
9	PiD	MFG	108.89	124.28	98.72
		STG	56.19	28.76	35.61
		ACG	1.37	1.37	0.00
10	PiD	MFG	109.76	96.02	105.48
		STG	55.14	54.81	45.20

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		ACG	102.73	89.03	101.37
11	PiD	MFG	131.03	133.42	131.41
		STG	102.73	77.12	52.79
		ACG	34.24	36.98	43.83
Wilcoxon signed-rank median difference (bias) >0			**vs.#2 p>0.3 vs.#3 p>0.6**	**vs.#3 p>0.2**	**-**
Case	**Diagnosis**	**Region**	**TDP %AO Investigator #1**	**TDP %AO Investigator #2**	**TDP %AO Investigator #3**
12	FLD-TDPC	MFG	0.48	0.42	0.32
		STG	0.70	0.49	0.88
		ACG	1.86	1.76	1.11
13	FLD-TDPC	MFG	0.47	0.41	0.46
		STG	0.28	0.34	0.37
		ACG	0.85	1.09	1.26
14	FLD-TDPC	MFG	0.07	0.14	0.34
		STG	1.48	1.06	1.11
		ACG	1.33	1.20	0.64
15	FLD-TDPC	MFG	0.66	0.59	0.59
		STG	0.16	0.11	0.21
		ACG	1.09	0.97	0.69
16	FLD-TDPC	MFG	0.58	0.47	0.91
		STG	0.43	0.57	0.67
		ACG	0.92	1.19	0.94
17	FLD-TDPC	MFG	0.15	0.22	0.28
		STG	0.39	0.44	1.25
		ACG	0.64	0.81	0.96
	FLD-TDPC	MFG	0.12	0.16	0.07

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18		STG	0.60	0.60	0.33
		ACG	0.90	0.91	0.57
19	FLD-TDPC	MFG	0.28	0.25	0.21
		STG	0.44	0.42	0.59
		ACG	0.49	0.67	0.52
20	FLD-TDPC	MFG	1.35	1.18	2.12
		STG	0.60	0.63	0.93
		ACG	1.41	1.09	1.67
21	FLD-TDPC	MFG	0.04	0.04	0.02
		STG	0.10	0.14	0.17
		ACG	0.10	0.25	0.07
Wilcoxon signed-rank median difference (bias)>0			**vs. #2 p>0.2 vs. #3 p>0.2**	**vs. #3 p>0.2**	**-**

Supplementary Table 4. Negative Control and Normal Control Data.

Case	Tau CI/mm^2	Tau %AO	TDP DN/mm^2	TDP %AO
PiD #1 MFC NC	0	0.0051	-	-
PiD #1 MFC NC	0	0.0002	-	-
PiD #1 MFC NC	0	0.0002	-	-
PiD #1 MFC NC	0	0.0003	-	-
PiD #1 MFC NC	0	0.0000	-	-
AVERAGE PiD NC	**0**	**0.0012**	-	-

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TDPC #1 MFC NC	-	-	1.4	0.0049
TDPC #2 MFC NC	-	-	3.2	0.0055
TDPC #3 MFC NC	-	-	1.4	0.0037
TDPC #4 MFC NC	-	-	0	0.0013
TDPC #5 MFC NC	-	-	0	0.0007
AVERAGE TDPC NC	-	-	**1.2**	**0.0032**
Normal #1 MFC	0	0.0064	0	0.0018
Normal #2 MFC	0	0.0030	0	0.0016
Normal #3 MFC	0	0.0388	4.3	0.0029
Normal #4 MFC	0	0.0002	0	0.0010
Normal #5 MFC	0	0.0028	0	0.0002
AVERAGE Normal	**0**	**0.0102**	**0.9**	**0.0015**

NC= negative control (IHC stain in absence of primary antibody).

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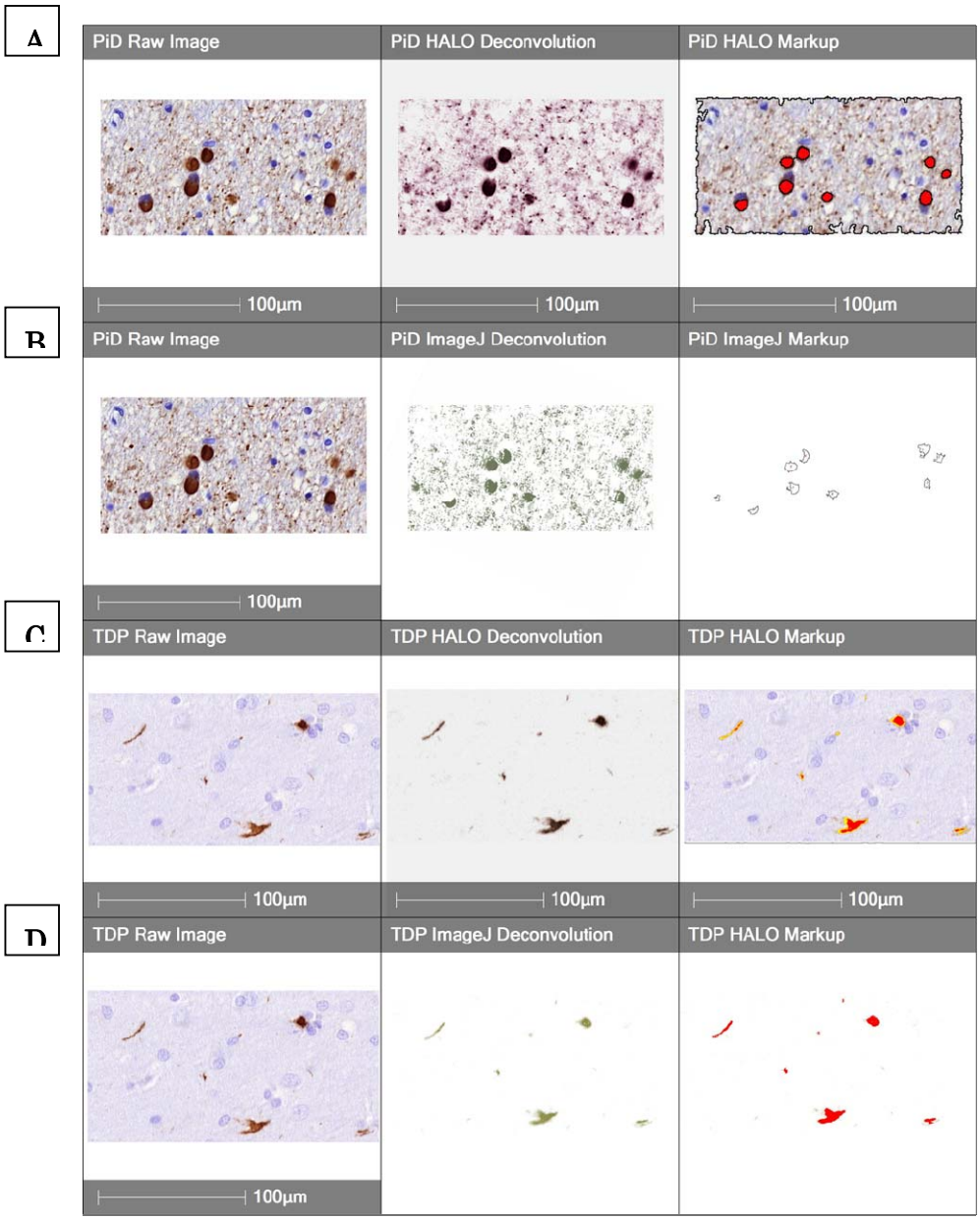

Supplementary Figure 3. Comparison of HALO and IMAGEJ digital image analysis platforms. Photomicrographs depict representative images from digital image analysis comparison between HALO (rows A, C) and IMAGEJ (rows C,D) for Tau CI object detection (rows A, B) and TDP %AO (rows C,D). Conversion of optimal algorithms into equivalent IMAGEJ input parameters results in a similar color deconvolution between platforms (column 2) and Tau CI detection (black and white outlines in row B column 3) and TDP %AO (red overlay in row D column 3) to HALO

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(red overlay in row A column 3 and yellow/orange/red overlay in row C column 3).

Supplementary Table 5. Image J Comparison Data

Case	Region	Tau CI/image HALO	Tau CI/image IMAGEJ	TDP %AO/image HALO	TDP %AO/image IMAGEJ
PiD #10	CING	7	9	-	-
PiD #3	STC	3	2	-	-
PiD#6	STC	4	4	-	-
PiD#11	CING	3	2	-	-
PiD#9	MFC	2	4	-	-
TDPC#7	STC	-	-	2.11	1.03
TDPC#6	CING	-	-	0.98	0.89
TDPC#4	MFC	-	-	1.07	1.07
TDPC#10	STC	-	-	1.35	1.29
TDPC#9	STC	-	-	2.18	2.31

* Average within-subject %CV for Tau CI detection=24.3%, TDP %AO= 12.5%