Non-invasive assessment of urinary exfoliated proximal tubule cells using multispectral autofluorescence imaging and machine-learning to identify between histopathological causes of kidney transplant complications

LIGHTnLOO

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2025 Young Investigator Award Session, Sept 1st 2025

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- Kidney Biopsy remains the gold-standard for diagnosis of kidney transplantation complications, but it is invasive
- Various serum and urine markers to achieve similar aims have been proposed over the years, but none fully convincing in terms of accuracy and precision
- Proximal tubule cells form >50% of the kidney mass; most likely the cell type to inform most about kidney status
- We considered a novel alternative proximal tubule cell isolation and cell assessment technique based on Artificial Intelligence as a diagnostic option for transplant complications

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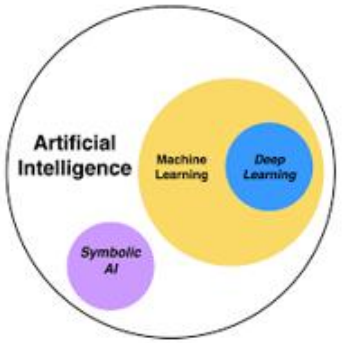
Extract PTCs with anti-CD13 and SGLT2 antibodies (Mahbub et al., 2021)

200ml bottle

Hyperspectral Microscopy



AI-based feature analysis

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- **Acute Tubular Necrosis**

10 patients, 51 proximal tubule kidney cells

vs

- **Graft Rejection**

10 patients, 60 proximal tubule kidney cells

vs

- **Interstitial Fibrosis and Tubular Atrophy (IFTA)**

10 patients, 50 proximal tubule kidney cells

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Table 1. Baseline demographic and clinical characteristics of the study groups (*n*=30)

COLUMN HEADING	ATN (*n*=10)	Rejection (*n*=10)	IFTA (*n*=10)	*P* Value[a]
Sex (male), *n* (%)	7 (70)	8 (80)	5 (50)	0.350
Age (yr), mean (SD)	55.0 (8.4)	39.1 (9.4)	54.1 (13.2)	0.028[b]
No. of days from transplant to biopsy, median (IQR)	5.0 (2.5)	96 (137.5)	1939 (1508.5)	<0.001[b]
eGFR (ml/min per 1.73 m^2), median (IQR)	33.5 (20)	34.5 (9.8)	26.5 (6.5)	0.261
uACR, median (IQR)	45 (20)	37 (31.8)	82 (74)	0.112
Albuminuria, *n* (%)	Micro: 8 (80) Macro: 2 (20)	Micro: 9 (90) Macro: 1 (10)	Micro: 5 (50) Macro: 5 (50)	0.110
Serum creatinine (μmol/L), median (IQR)	179.5 (160.3)	196 (27.8)	238 (91)	0.814
Distribution of allograft biopsy diagnosis, *n* (%)	All ATN	Acute ABMR: 2 (20) Acute TCR: 1 (10) Chronic active ABMR: 2 (20) Recurrent ABMR: 1 (10) Chronic TCR: 2 (20) Recurrent TCR: 1 (10) Mixed chronic BCR and TCR: 1 (10)	Chronic allograft nephropathy: 2 (20) CNI toxicity-associated IFTA: 4 (40) Diabetic nephropathy: 1 (10) Progressive fibrosis in the absence of immunologic challenge: 3 (30)	N/A

ABMR, antibody-mediated rejection; ACR, albumin-creatinine ratio; ATN, acute tubular necrosis; BCR, B-cell rejection; CNI, calcineurin inhibitor; IFTA, interstitial fibrosis and tubular atrophy; IQR, interquartile range; TCR, T-cell rejection.
[a]*P* values are calculated using the ANOVA test (if normally distributed) or Kruskal-Wallis test (if the distribution is nonparametric) for continuous variables, and the chi-squared test or Freeman-Halton extension of the Fisher exact test accounting for sparsely distributed data for categorical variables.

Wu et al., *Kidney360*, 2025

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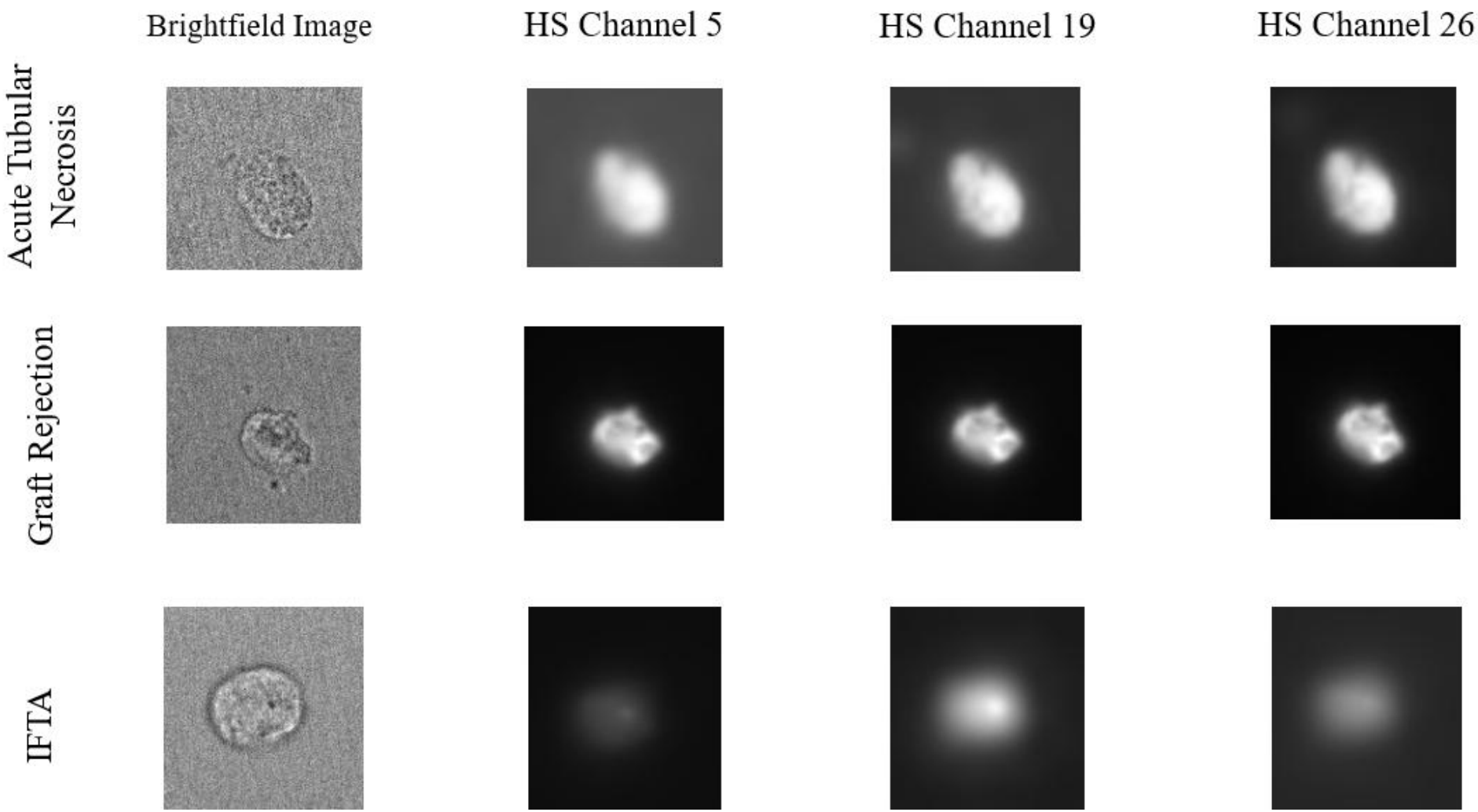

AutoGluon-based Analysis

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AutoGluon-Tabular: Robust and Accurate AutoML for Structured Data

Nick Erickson [*1] Jonas Mueller [*1] Alexander Shirkov [1] Hang Zhang [1] Pedro Larroy [1]
Mu Li [1] Alexander Smola [1]

Erickson et al., *arXiv*, 2020

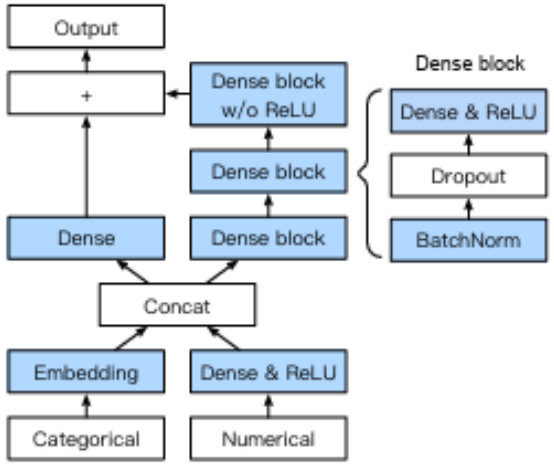

Figure 1. Architecture of AutoGluon's neural network for tabular data composed of numerical and categorical features. Layers with learnable parameters are marked as blue.

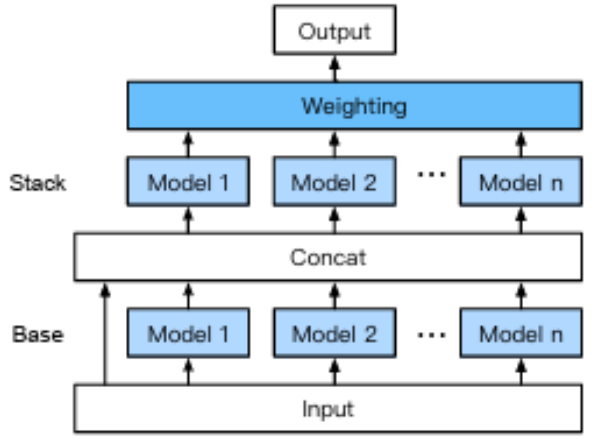

Figure 2. AutoGluon's multi-layer stacking strategy, shown here using two stacking layers and n types of base learners.

Algorithm 1 AutoGluon-Tabular Training Strategy (multi-layer stack ensembling + n-repeated k-fold bagging).

Require: data (X, Y), family of models $\mathcal{M}$, # of layers L

Preprocess data to extract features
for $l = 1$ **to** L **do** {Stacking}
  for $i = 1$ **to** n **do** {n-repeated}
    Randomly split data into k chunks $\{X^j, Y^j\}_{j=1}^k$
    for $j = 1$ **to** k **do** {k-fold bagging}
      for each model type m in $\mathcal{M}$ **do**
        Train a type-m model on X^{-j}, Y^{-j}
        Make predictions $\hat{Y}_{m,i}^j$ on OOF data X^j
      end for
    end for
  end for
  Average OOF predictions $\hat{Y}_m = \{\frac{1}{n}\sum_i \hat{Y}_{m,i}^j\}_{j=1}^k$
  $X \leftarrow$ concatenate$(X, \{\hat{Y}_m\}_{m \in \mathcal{M}})$
end for

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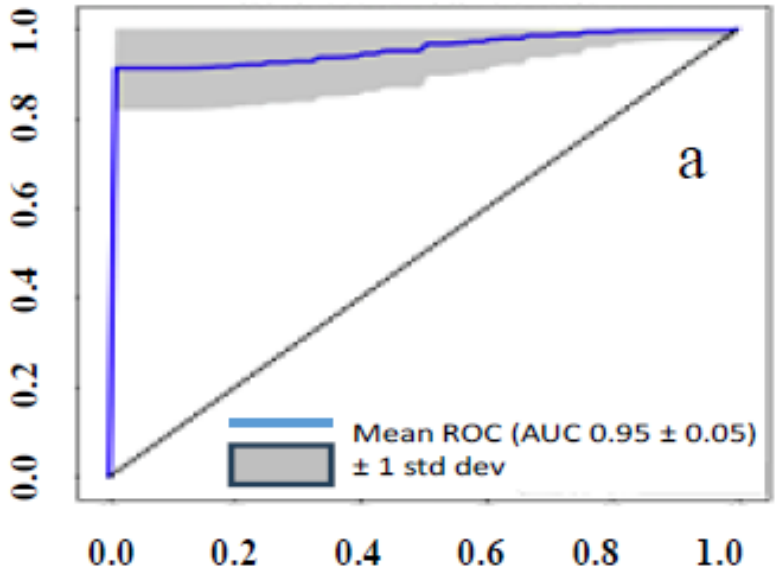
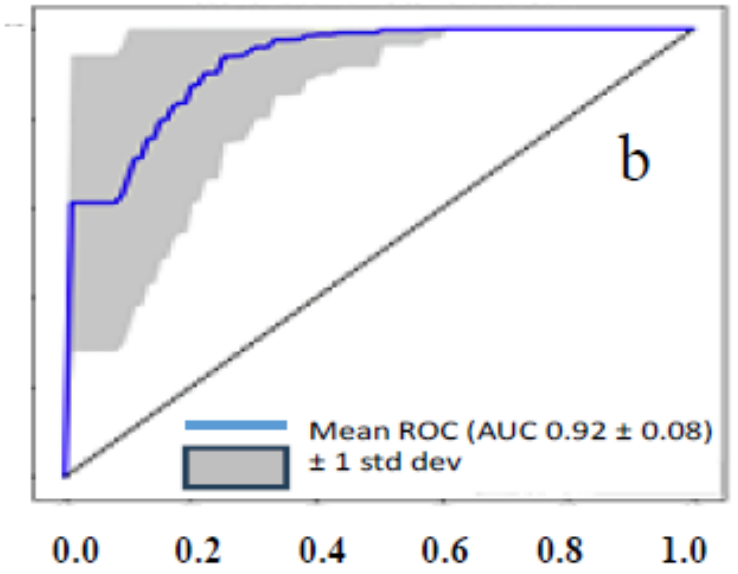
ATN vs Rejection



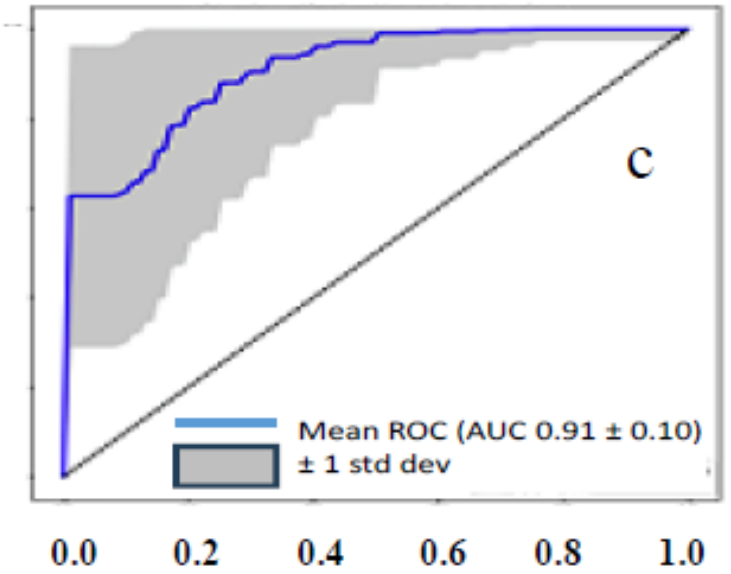
AUC 0.95 (95%CI 0.90-1.00)

ATN vs IFTA



AUC 0.92 (95%CI 0.84-1.00)

Rejection vs IFTA



AUC 0.91 (95%CI 0.81-1.00)

Summary of Study Results

- We demonstrated measurement of urinary exfoliated PTC autofluorescence could be used to differentiate between ATN versus graft rejection and non-rejection IFTA in kidney transplant recipients

- Our proposed ‘liquid biopsy’ methodology has tremendous translational potential going forwards as a companion diagnostics tool in transplantation

Ongoing work…

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- Continue building up patient numbers across the groups to perform subgroup analysis

e.g. in the 'IFTA' group – we are comparing subgroups between Calcineurin Inhibitor Toxicity vs BK Virus Nephropathy vs Progressive fibrosis in the absence of immunological challenge vs Chronic allograft nephropathy etc.

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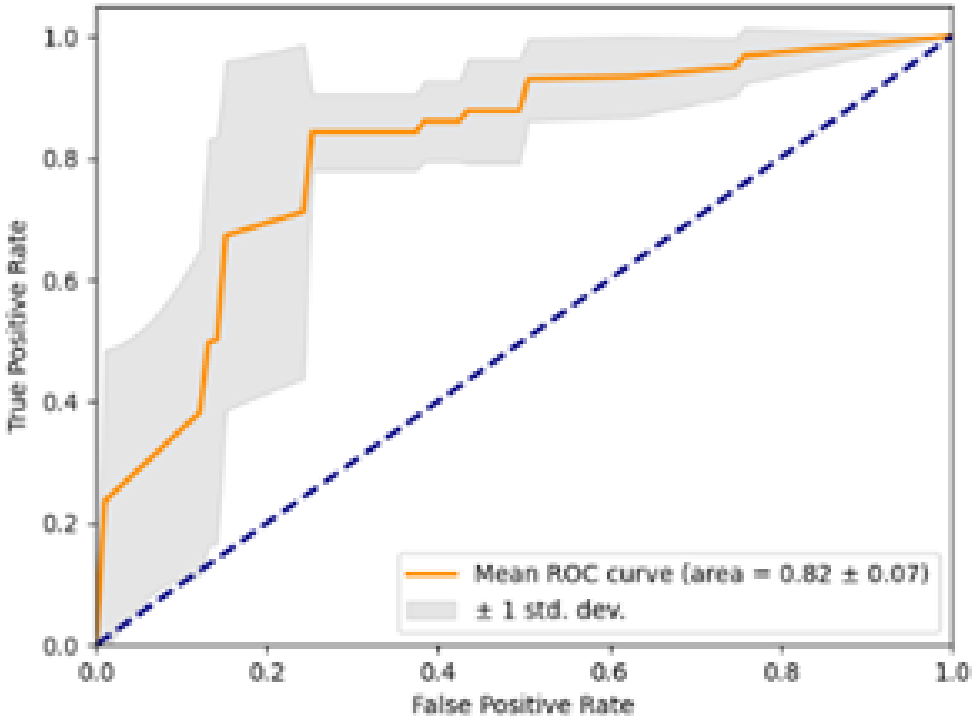
T-cell rejection (n = 10) vs Antibody-mediated rejection (n =10)



AUC value 0.82 ± 0.07

Ongoing work…

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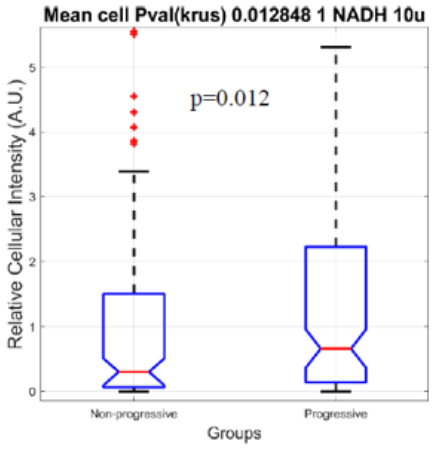
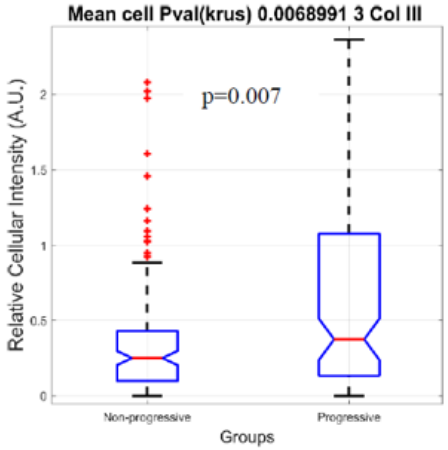
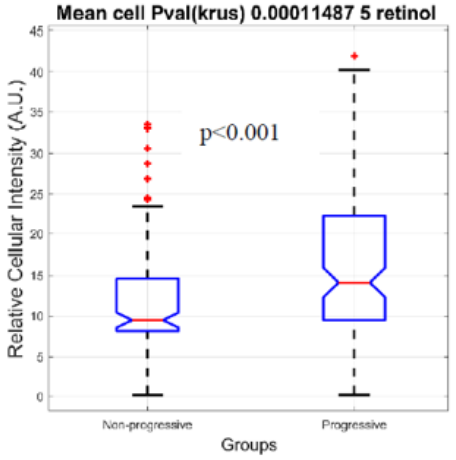
- To perform spectral unmixing to determine the biological fluorophores which differentiate between the autofluorescence signals emitted in PTCs across the groups



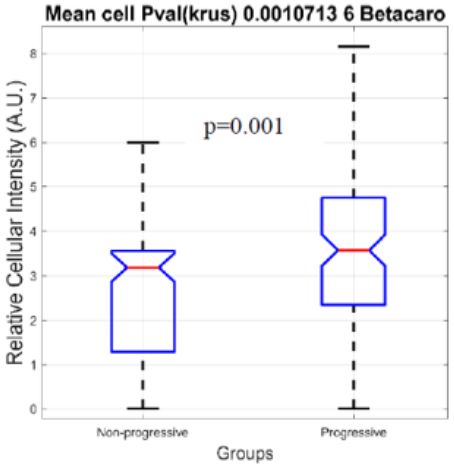
NADH



Type III Collagen



Retinol



Beta-carotene

Spectral unmixing data comparing between progressive CKD (*n = 15*) vs non-progressive CKD groups (*n = 15*)
(unpublished data)

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Non-invasive Assessment of Urinary Exfoliated Proximal Tubule Cell Multispectral Autofluorescence May Differentiate Between Causes of Kidney Transplant Dysfunction

Wu, Henry H.L.[1,2,3,a]; Lang, Yandong[2]; Handley, Shannon[2]; Knab, Aline[2]; Agha, Adnan[2]; Tian, Yuan[2]; Bhargava, Akanksha[2]; Goldys, Ewa M.[*,2]; Pollock, Carol A.[*,1,3]; Saad, Sonia[*,1]

Author Information

Kidney360 ():10.34067/KID.0000000879, June 20, 2025. | *DOI:* 10.34067/KID.0000000879

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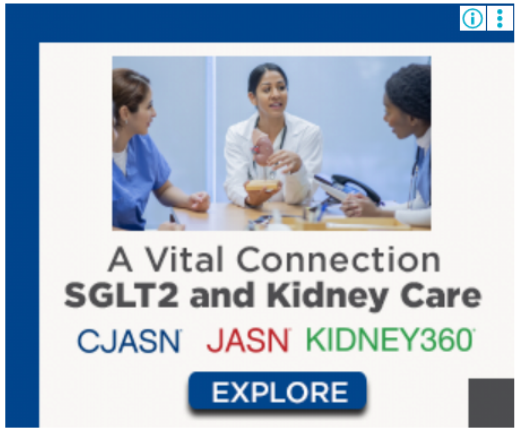

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