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**FP293** DETERMINATION OF OXYGEN TENSION IN TUBULAR EPITHELIAL CELLS USING PHOSPHORESCENCE LIFETIME IMAGING MICROSCOPY

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**INTRODUCTION AND AIMS:** Although tubulointerstitial hypoxia has potential to accelerate chronic kidney disease (CKD) progression, there is no established quantitative technique which can assess oxygen tension inside tubular or interstitial cells with high spatial resolution. We have applied phosphorescence to renal tubular oxygen tension assessment using the unique character of phosphorescence that its lifetime depends on oxygen tension (Sci Rep 2015). In this research, we use phosphorescence lifetime imaging microscopy (PLIM) in order to examine the oxygen tension in cortical tubular epithelial cells.

**METHODS:** We used BTPDM1, a lipophilic phosphorescent dye, as an oxygen sensor. PLIM measurements were performed with an inverted confocal microscope. FITC-conjugated Lycopersicon Esculentum lectin and 4kDa dextran were used as counterstaining of endothelial cells and urinary space, respectively. Values are shown as mean and range from -1 S.D. to +1 S.D.

**RESULTS:** PLIM and counterstaining of vasculature were successfully performed in renal cortex, and the observable area was within 10μm of depth. The tubules can be divided into two subgroups according to phosphorescence lifetime. Administration of urinary excreted fluorescent dye, FITC-conjugated 4kDa dextran, revealed that tubules which display shorter phosphorescence lifetime, namely in higher oxygen tension, are positioned in upstream of the other tubules; in other words, tubules of S1 segments are in higher oxygen tension as 54 (44-67) mmHg and tubules of S2 segments are in lower oxygen tension as 45 (36-55) mmHg. These values were consistent with the result of needle microelectrode. The phosphorescence lifetime of both S1 and S2 segments became elongated and shortened in 15% O2 inhalation and reoxygenation, respectively, indicating this method indeed reflects oxygen tension. We applied this PLIM to unilateral ureteral obstruction (UUO) model and found that UUO kidneys are more hypoxic than sham operation kidney, although the absolute value of pO2 in UUO kidney cannot be assessed.

**CONCLUSIONS:** We established a method which can assess tubular segment-specific oxygen status in living mice. By PLIM with BTPDM1, the oxygen tension of S1 segment was indicated to be higher than S2 segment.