Structured illumination microscopy and automatized image processing as a rapid diagnostic tool for podocyte effacement

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The normal morphology of podocyte foot processes is obligatory for proper renal filtering function. The most common cause for a nephrotic syndrome in children is minimal change disease (MCD). In the case of MCD, the right diagnosis is found by transmission electron microscopy as conventional light microscopy of kidney biopsies only display minimal or even no pathological alterations at all. In MCD, the only pathologic change is the effacement of podocyte foot processes which leads to loss of the size-selectivity of the glomerular filtration barrier and massive proteinuria. A problem is that electron microscopy requires time consuming preparation and evaluation which leads to a long duration between the diagnosis of nephrotic syndrome and the right diagnosis MCD.

Lately, a variety of superresolution microscopy techniques have been developed which all can exceed the optical resolution limit of 200 µm. One of those techniques is structured illumination microscopy which allows a twofold increase of resolution in x, y and z direction and works with conventional tissue processing techniques and fluorophores.

As a proof of principle and to test whether our technique could be used as a diagnostic tool for MCD, we measured a mean foot process width of 0.249±0.068 µm in healthy kidneys and a significant higher mean foot process width of 0.675±0.256 µm in MCD patients.

We then hypothesized that the density of the slit diaphragm on glomerular capillaries could be used equivalent for the diagnosis of effacement. To prove that, we programmed an ImageJ plugin that automatically measures the density of the slit diaphragm. We measured a mean of 3.099±0.268 µm/µm^2 in healthy subjects and 1.825±0.493µm/µm^2 in MCD patients. As both measurements were directly correlated (R^2=0.91), we concluded that our approach is a legitimate method with various advantages for the diagnosis of foot process effacement.

Here we describe a method for the superresolution-visualization of podocyte foot processes and the slit diaphragm using structured illumination microscopy on conventionally processed and nephrin-stained human tissue samples in combination with automatic digital image processing. Due to its diverse advantages, we propose this technique as an addition to the renal histopathological toolkit.