Fully automated evaluation of total glomerular number and capillary tuft size in murine nephritic kidneys using lightsheet microscopy.

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Abstract

The total number of glomeruli is a fundamental parameter of kidney function, but very difficult to determine using standard methodology. Here we individually counted all glomeruli in murine kidneys and sized their capillary tufts by combining in vivo fluorescence labeling of endothelial cells, a novel tissue-clearing technique, lightsheet microscopy and automated registration by image analysis. Total hands-on time per organ was below one hour and automated counting/sizing was finished in less than three hours. For clearing we introduce Ethyl-cinnamate (Ethyl-3-phenylprop-2-enoate) as novel and first non-toxic solvent-based reagent, that can be handled without specific safety measures. Ethyl-cinnamate rapidly clears any organ including calcified bone thereby maintaining fluorescence of proteins and immunohistochemical labels over weeks. Using Ethyl-cinnamate-cleared kidneys we also quantified the average creatinine-clearance per glomerulus. We show that this parameter declines already in the first week of

experimental nephrotoxic nephritis, whereas glomerular numbers start doing so only later. Our approach delivers fundamental parameters of renal function and due to its ease and speed is suitable for high-throughput analysis. Thus it should greatly facilitate the study of various types of kidney disease.

Beteiligte Forschungseinheiten

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