Contact Information

These images were taken in the laboratory of Brian Cobb at Case Western Reserve University School of Medicine (brian.cobb@case.edu; http://case.edu/medicine/pathology/labs/cobb-lab/) using the indicated lectin supplied by Vector Laboratories. For any questions about the methods or images, please contact Dr. Cobb.

Methods

Tissues

Resting C57Bl/6 mice between 8 and 12 weeks of age were sacrificed by CO_2 inhalation. Tissues were removed, formalin fixed (lungs were inflated) and embedded in paraffin for sectioning.

Staining

Slides were incubated in two 10 minute Xylene washes to remove any Paraffin and other mounting particulates, and were then re-hydrated using the following ethanol (EtOH):H_2O gradient (each are 3 minutes): two washes in 100% EtOH, one 75% EtOH wash, one 50% EtOH wash, two Tris-buffered saline (TBS with 0.05% tween, pH 7.5) washes. Epitope retrieval was performed by washing in 100°C 10 mM citrate buffer (10 mM sodium citrate, 2 mM EDTA, pH 6.2), for 3 minutes, then washed 3 times in 1 mg/mL sodium borohydride in TBS at 4°C to reduce auto-fluorescence, followed by a brief TBS wash.

Blocking was performed using 1X Carbohydrate-free blocking solution (Vector Labs, Carbo-Free Blocking Solution 10x concentrate, Cat# SP-5040) for 30 minutes with gentle rocking. Lectin was diluted in blocking solution and added to tissue samples following blocking and incubated for 1 hour in the dark with gentle rocking at room temperature. Samples were washed 3 times in TBS for 10 minutes each. Two drops of mounting media (Vector Labs, VectaShield Hardmount, Cat# H-1400) were added to each tissue section, and an appropriately sized cover slip was used to seal the tissue. Slides were allowed to dry at room temperature in the dark for ~10-15 minutes before being moved to 4°C until imaged.

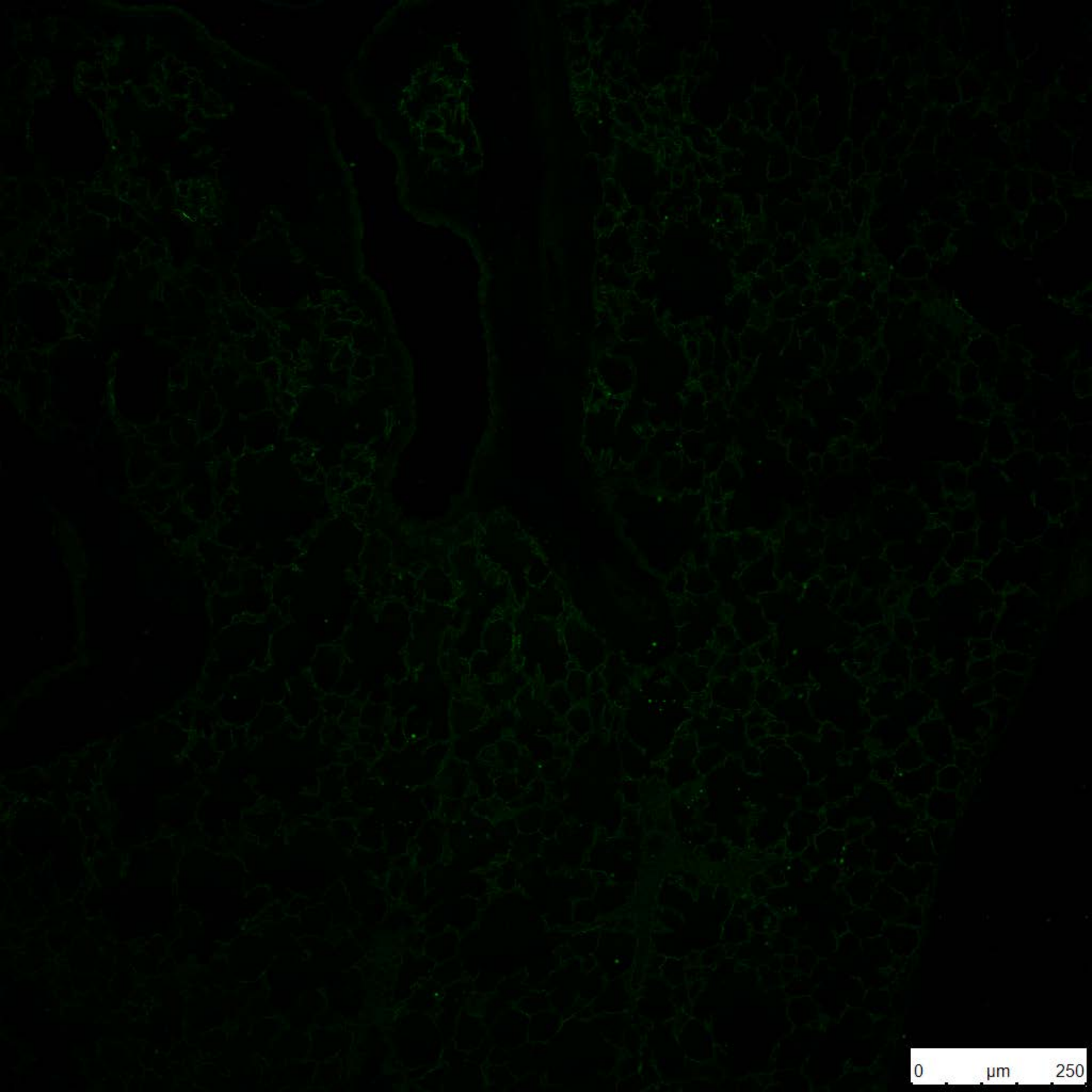
Imaging

All images were collected on a Leica SP5 Laser Scanning Confocal Microscope. The approach was to use the brightest tissue among the four (liver, spleen, lung, and kidney) to set the laser and detector strengths, and then to use those *same settings* for all four tissues. Thus, signal brightness is comparable between tissues within this dataset.

Lectin

Fluorescein-conjugated ECL, Lot #ZB0508

Lung 10x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µm
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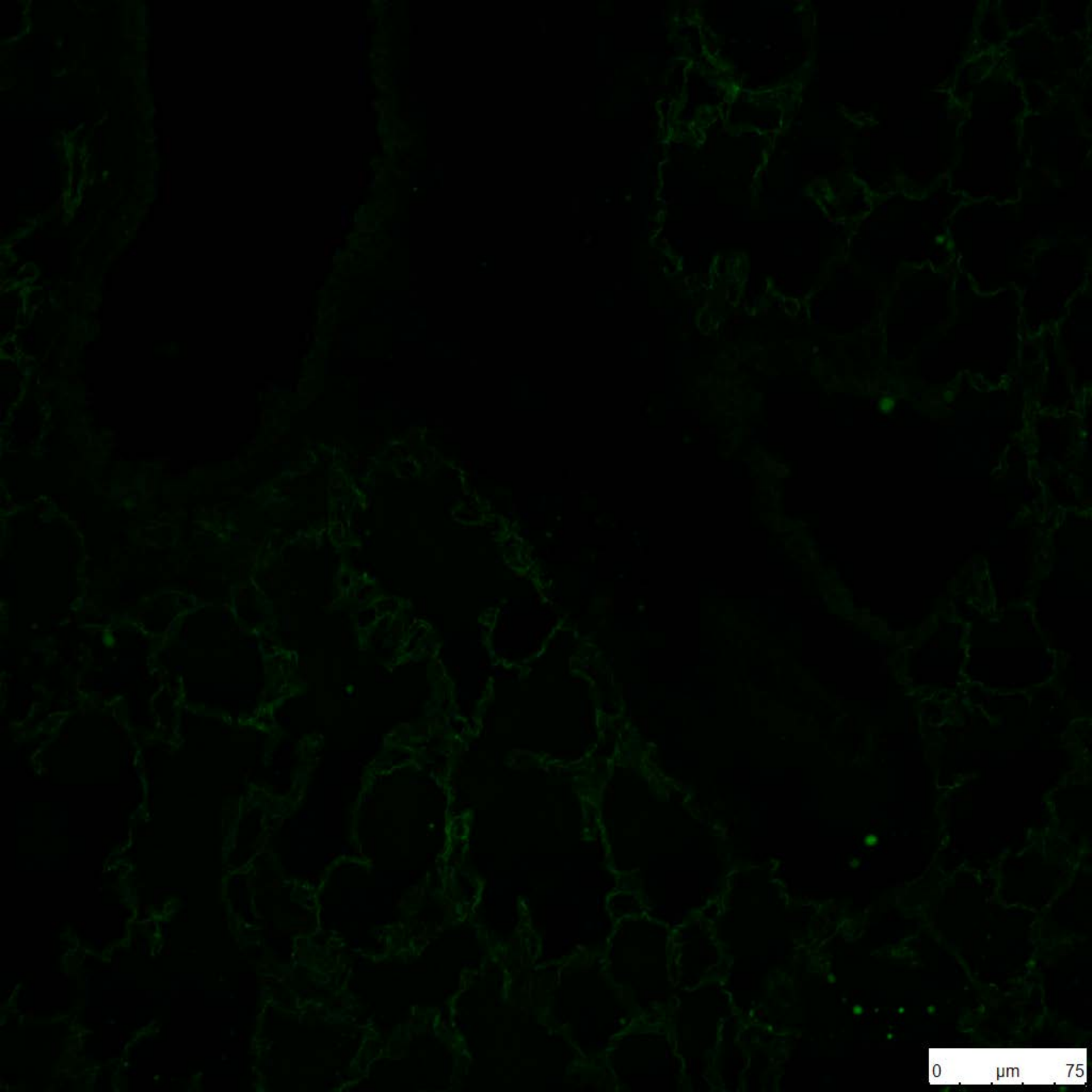
Lung 30x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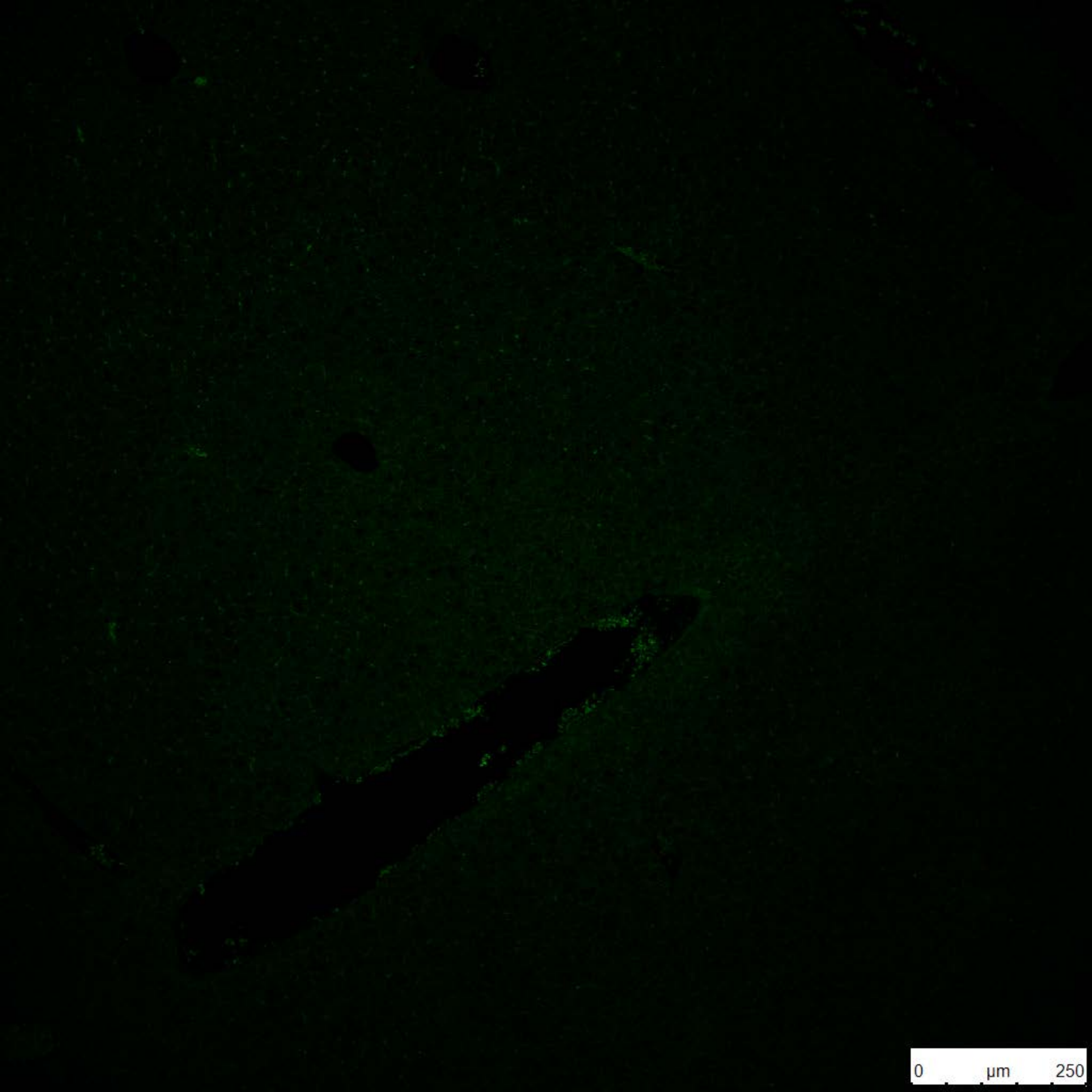
µm
75

Liver 10x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µm
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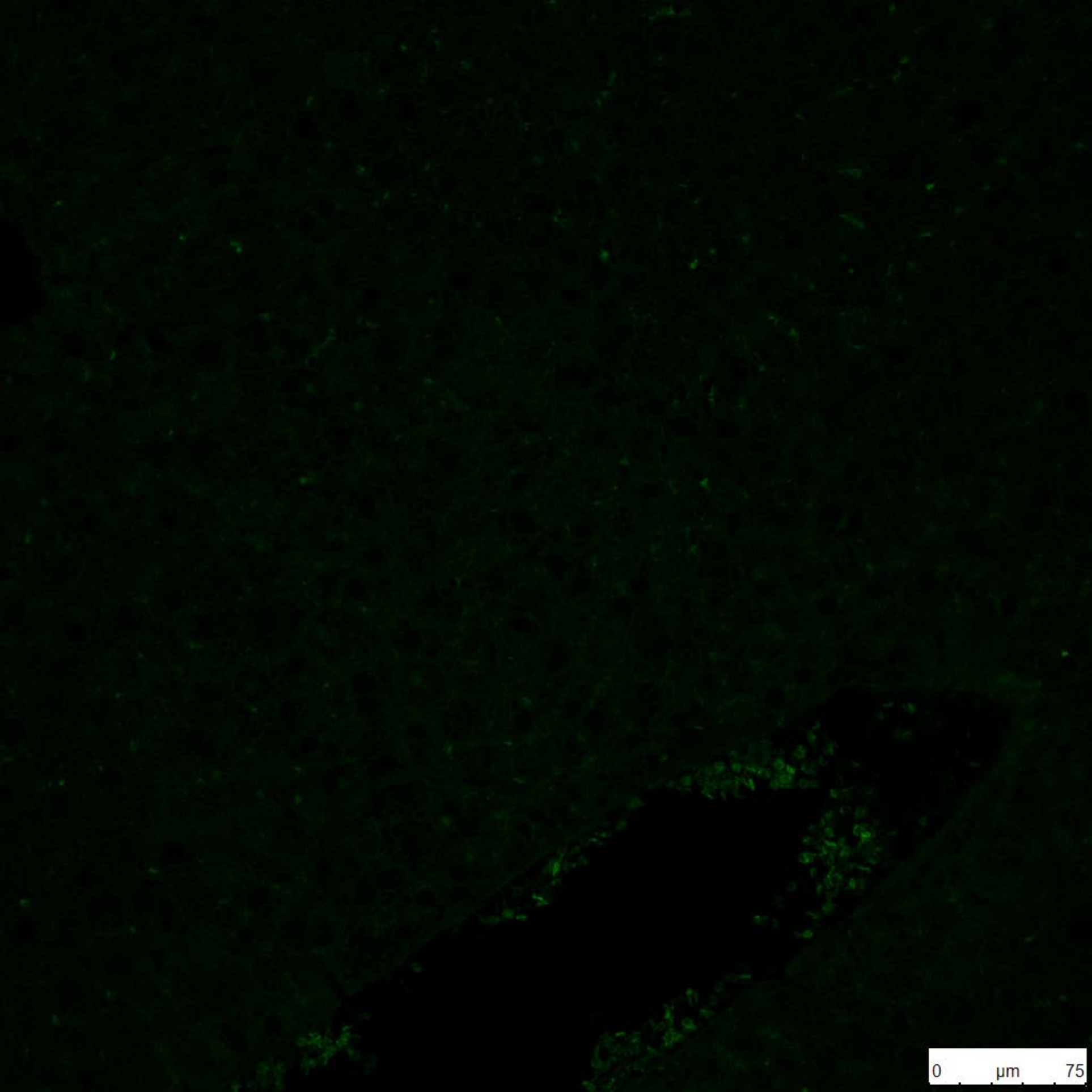
Liver 30x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µm
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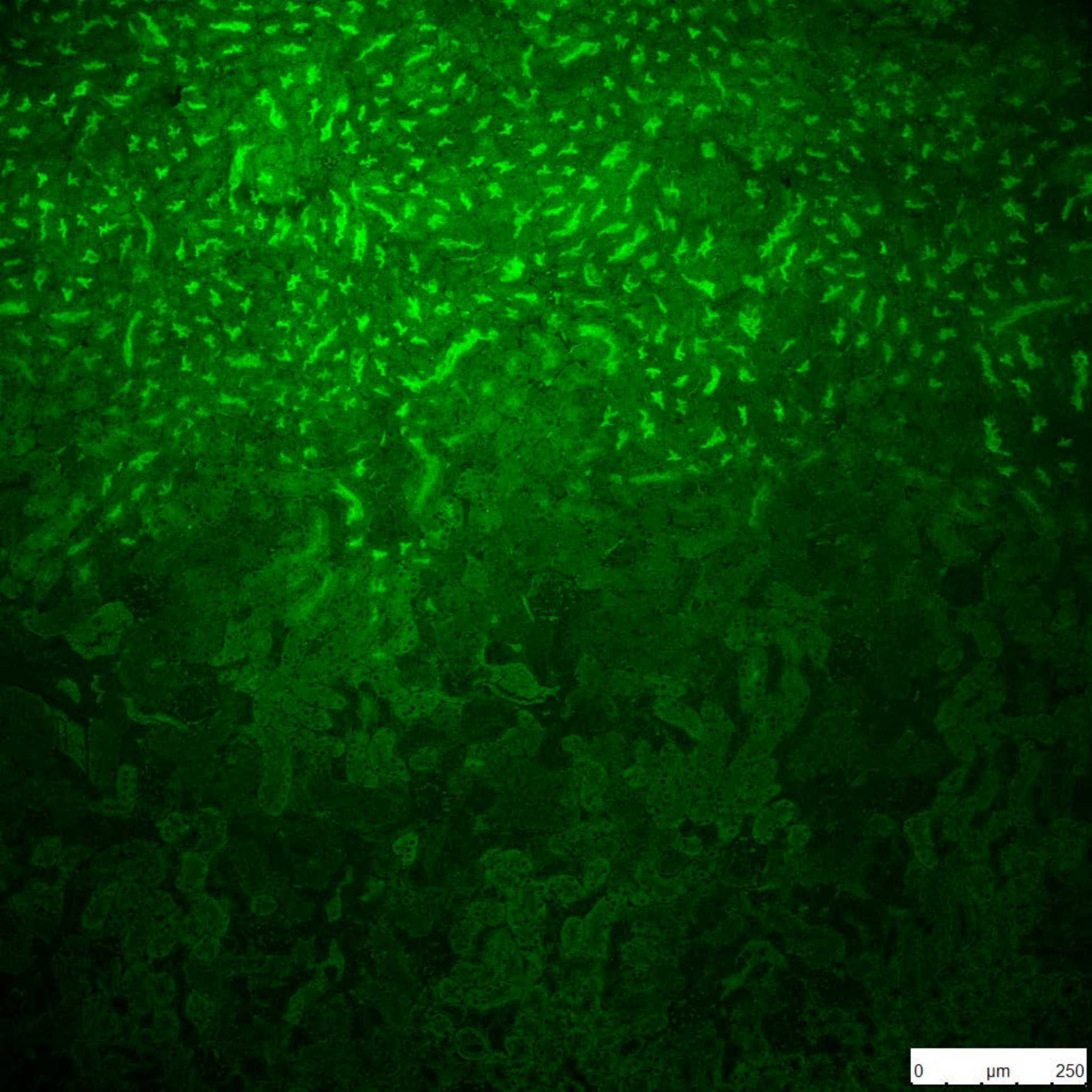
Kidney 10x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µm
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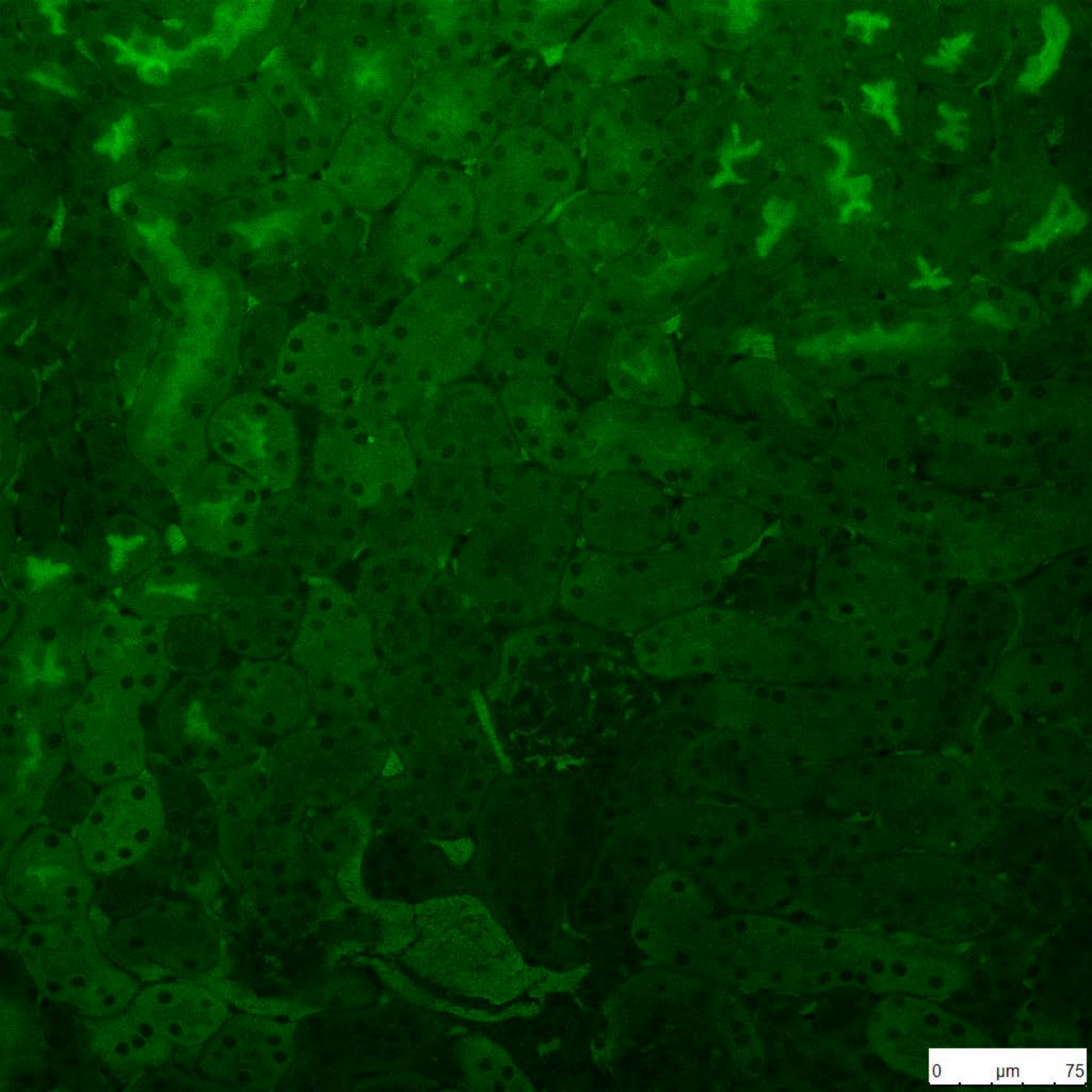
Kidney 30x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µm
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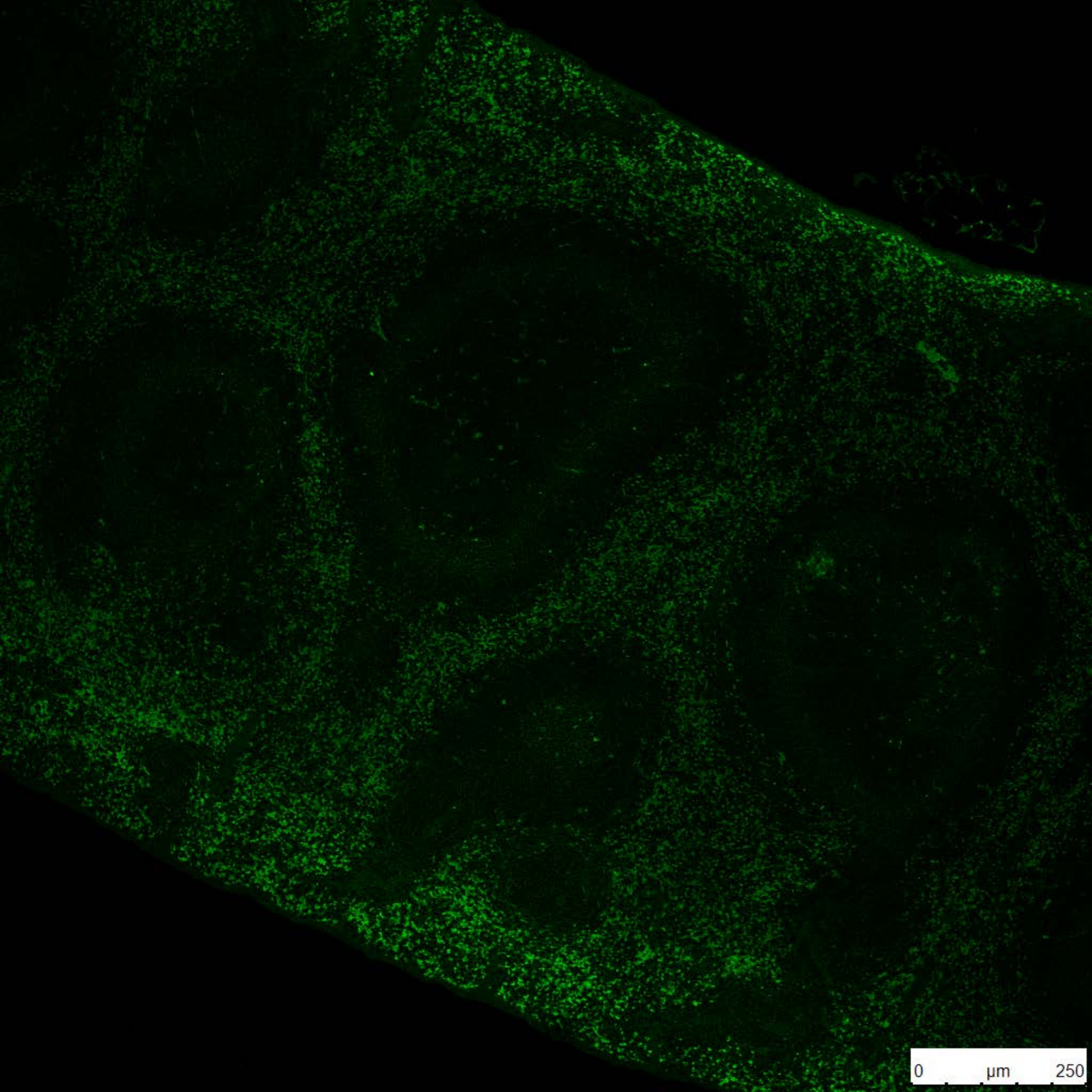
Spleen 10x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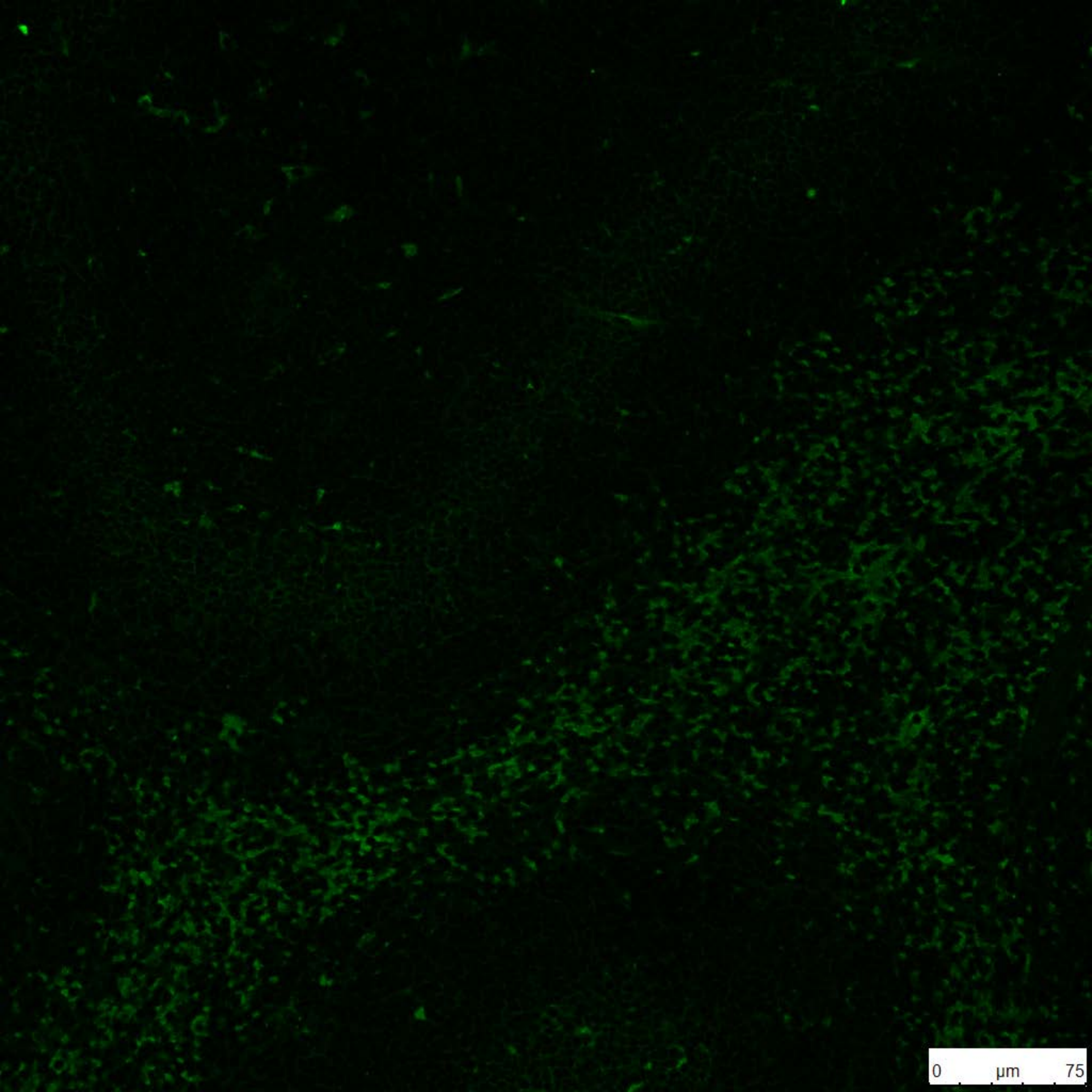
Spleen 30x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µm
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