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## **Molecular imaging in the eye**

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**Abstract**

Molecular imaging is playing an increasingly powerful role in elucidating pathophysiological pathways, in advancing drug discovery and in deciphering developmental processes. Multiple modalities, including optical imaging, ultrasound, nuclear imaging, computed tomography and various techniques of magnetic resonance imaging (MRI), are now being used to obtain fundamental new insights at the cellular and molecular level, both in basic research, using animal models, and in clinical studies.

In permitting unique optical access, the eye is particularly well suited for molecular imaging, e.g. transgenic mice in which the fractalkine receptor is rendered intrinsically fluorescent to allow for in-vivo monitoring of myeloid immune cells within the retina and choroid by scanning laser ophthalmoscopy (SLO). Retinal cell apoptosis can be assessed by intravitreal injection of fluorescent-labelled annexin 5 in vivo, using a similar SLO technique. Intravital microscopy also allows visualization of CD11c-positive dendritic cells in transgenic mice expressing yellow-fluorescent protein in these immune cells. Adoptive transfer of fluorescent-labelled transgenic T-cells enables visualization of infiltration by specific T cells into various eye compartments. On the other hand, functional imaging can be provided by new magnetic resonance (MR) methodologies: The deuterium MRI and diffusion MRI analysis techniques permit dynamic studies of water movement in animal eyes. MRI also enables pharmacokinetic studies on ocular drug delivery and detects biomarkers for treatment efficacy in retinopathies.

Undoubtedly, these and further molecular imaging techniques currently being developed will have fundamental impact on experimental and clinical ophthalmology

and thus on our understanding of eye disease and development of therapy in general.

**Key words**

Molecular imaging, labelling, cellular imaging, in-vivo imaging, MRI, SLO

## **Introduction**

The ability to measure biochemical and molecular processes is key to achieving advances in both basic and clinical ophthalmology. Functional and molecular imaging allows in-vivo assaying of biological and physiological processes, thus providing new insight into pathomechanisms of underlying ophthalmological disease and the impact of treatment modalities. The monitoring of drug targeting may especially play a key role in the development of new treatment options beyond anti-VEGF therapy for proliferative eye disease.

This review describes different imaging techniques and devices used in molecular imaging of the eye, as well as potential imaging tools and targets.

## **IMAGING TECHNIQUES AND DEVICES**

### **Optical imaging**

The use of visible light to examine intraocular processes can be considered the oldest form of imaging in ophthalmology. The unique optical properties of the eye allow direct microscopic observation of the anterior and posterior segment. Optical molecular imaging technologies use light emitted through fluorescence or bioluminescence. In fluorescence imaging, light of the excitation wavelengths must penetrate tissues to reach a targeted reporter molecule carrying a fluorochrome, resulting in the emission of light of usually lower wavelength that can be registered by a CCD camera.

Fluorescent proteins, such as cyan, green or yellow fluorescent protein (CFP, GFP and YFP, respectively) can be introduced into cells of choice by transgenic technology. This technology has greatly facilitated studies on mononuclear phagocytes in vivo using CX<sub>3</sub>CR1<sup>GFP/+</sup> knockin mice. In these animals, one copy of the fractalkine receptor (CX<sub>3</sub>CR1) gene has been replaced by GFP, leaving the endogenous promoter intact. This modification renders all cells expressing the fractalkine receptor, such as microglia cells (μGCs), dendritic cells (DCs) and macrophages (MΦs), intrinsically fluorescent [1]. Paques and co-workers have shown the presence of μGCs in the retina of CX<sub>3</sub>CR1<sup>GFP/+</sup> mice by scanning laser ophthalmoscopy (SLO) [2]. This technique also permits visualization of DCs and MΦs in the fundi of viable CX<sub>3</sub>CR1<sup>GFP/+</sup> mice, and thus allows kinetic studies in the same animals. Consequently, we investigated the in-vivo response of these mononuclear phagocytes, i.e. DCs and MΦs, in the retina and choroid after laser-induced injury, to better understand the role of these cells in inflammatory eye disease [3]. We examined CX<sub>3</sub>CR1<sup>GFP/+</sup> mice by confocal SLO (cSLO) after argon laser coagulation. cSLO was complemented by fluorescence microscopy of retinal flat-mounts and eye cryosections to study cell morphology and location, and by multicolour flow cytometry to determine cell numbers and identity. It was shown that the retina of healthy reporter mice featured abundant fluorescent μGCs. By 60 min after laser injury to the fundus, these cells had accumulated and migrated laterally towards the site of injury. Distinctly shaped fluorescent cells accumulated within laser spots and were identified by flow cytometry and immunofluorescence microscopy as DCs and MΦs in retina and choroid. DCs rapidly disappeared from the retina, whereas MΦs stayed longer. Choroidal infiltrates were detectable as long as 35 days after laser injury, particularly in larger spots resulting from higher laser intensity. Therefore, cSLO allows detailed

investigation of the in-vivo response of mononuclear phagocytes to laser injury of the fundus (Fig. 1) [3].

In bioluminescence imaging, light of 510--670 nm is emitted through an enzymatic reaction involving luciferin and luciferase. Certain filters, such as those used by fluorescein or indocyanine green angiography, allow the detection of specifically fluorescent structures. As in fluorescence imaging, numerous transgenic animals have been generated that express various types of luciferase under different promoters whose expression in disease models can be measured after luciferin injection. Imaging stations have been developed that allow detection of even faint light emission from within the body of experimental animals. This method is particularly powerful when long emission wavelengths are employed, because these penetrate living tissues much better. Non-invasive time-course analyses have therefore become possible, and could theoretically be of great use in ophthalmology as well as in other fields.

Inflammation and tracing of inflammatory cells has been a key topic in molecular imaging in recent years. Using an established model of ocular inflammation, endotoxin-induced uveitis, Miyahara and colleagues visualized the rolling and adhesive interaction of fluorescent microspheres conjugated to recombinant P-selectin glycoprotein ligand-Ig (rPSGL-Ig) in the choriocapillaris by means of SLO. At inflammatory sites, endothelial cells expressed adhesion molecules that caused leukocyte recruitment. Leukocyte rolling, the first step in the recruitment process, is mediated primarily by the interaction between P-selectin on the endothelial surfaces and its main ligand, P-selectin glycoprotein ligand-1 (PSGL-1), expressed on the

leukocyte surface. PSGL-1-conjugated fluorescent microspheres therefore allow specific detection of endothelial P-selectin expression in vivo, and thus non-invasive assessment of endothelial injury [4]. Leukocyte dynamics itself has been investigated in rodent models of experimental uveitis [5-9]. Acridine orange digital fluorography revealed leukocytes rolling in the retina of rats in experimental autoimmune uveoretinitis [5]. Acridine orange solution was injected continuously through a tail vein at 1 ml/min. Retinal images were generated by a scanning laser ophthalmoscope connected to a computer-assisted image analysis system. Acridine orange binds to DNA and RNA, and the spectral properties of acridine orange--DNA complexes are very similar to those of sodium fluorescein, with a 448 nm excitation maximum and an emission maximum of 522 nm. Results show that leukocyte--endothelium interaction and extravascular infiltration in the retinal venous vasculature may play significant roles in the early stages of posterior segment inflammation [5]. Another method of investigating leukocytes in the retinal vasculature by SLO, reported by Xu and colleagues, involves injection of calcein-AM labelled T-cells into the tail vein of rodents [10]. Leukocyte dynamics can also be monitored in the iris stroma, limbus and choroid using intravital microscopy with an epifluorescent illumination microscope equipped with a black-and-white camera connected to a video capture card. Leukocytes were stained either with rhodamine G6 [6,8] or carboxyfluorescein-diacetate-succinimidyl ester (CFSE) [9] to monitor the iris and limbus, or SYTO-16 to visualize leukocytes in the choroid [8]. Interleukin-2 (IL-2), which is expressed upon stimulation of T-cells, commonly serves as T-cell activation marker. Becker and co-workers used enhanced GFP as a reporter gene for IL-2 expression. They showed by intravital microscopy that transgenic mice expressing GFP under the control of IL-2 regulatory elements can be used for in-vivo expression assays that allow detection of activated T-cells in the iris at multiple time points within the same animal with

experimental uveitis [7]. Transgenic reporter mice for numerous other cytokines exist, but have not yet been used in ophthalmology. Intravital microscopy has also been used for imaging dendritic cells in the cornea using transgenic mice that express YFP under control of the CD11c promoter (CD11c-YFP) [11].

Confocal SLO has also been used to visualize apoptosis of single nerve cells in the retina in vivo, in order to perform longitudinal studies of disease processes such as glaucoma [12]. This technique enables direct observation of single nerve cell apoptosis by using Alexa Fluor 488-labeled annexin 5 and a prototype Zeiss confocal scanning laser ophthalmoscope. It was possible to image changes in nerve cell apoptosis over hours, days and months. This revealed that effects depended on the magnitude of the initial apoptotic stimulus in several models of neurodegenerative disease in rat and primate. One of these studies investigated the neuroprotective effects of different glutamate modulation strategies, with a non-selective (MK801) and a selective (ifenprodil) NMDA receptor antagonist and a metabotropic glutamate receptor agonist (LY354740), in glaucoma-related in-vivo rat models of retinal ganglion cell (RGC) apoptosis. All strategies for glutamate modulation reduced staurosporine (SSP)-induced RGC apoptosis, with a combination of MK801 and LY354740 being the most potent [13]. The same group also demonstrated apoptosis in vivo after laser injury of the retina [14].

Further developments in cSLO technique yielded in-vivo retinal images at a cellular level. Adaptive optics SLO was used to image the retinal pigment epithelial (RPE) cells in patients with cone--rod dystrophy and bilateral progressive maculopathy [15]. "Adaptive optics" denotes a set of methods for measuring and compensating for the

aberration of individual eyes, consisting of trial lenses to correct sphere and cylinder, a Shack--Hartmann-based wavefront sensor to detect residual aberration and a deformable mirror to correct this residual aberration. Integrated into a scanning laser ophthalmoscope, lateral resolution of 2  $\mu\text{m}$  could be achieved, which enables imaging of RPE cells, cone photoreceptors [16], the flow of single white blood cells [16] and the lamina cribrosa [17]. Choi and co-workers have integrated adaptive optics into a fundus camera for imaging cone photoreceptors in patients with retinal dystrophies and optic neuropathy [18,19].

Retinal ganglion cells (RGCs) have been imaged in vivo by a confocal scanning laser microscope using different transgenic zebra fish lines in which fluorescent protein was expressed in RGCs. By means of in-vivo time-lapse imaging Mumm and co-workers were able to obtain new insight into how RGCs establish contacts with other neurons [20]. Simultaneous imaging of RGCs and subpopulations of presynaptic amacrine interneurons over a period of days revealed that RGC dendrites targeted amacrine plexuses [20]. The dynamic behaviour of axons can also be studied by intraretinal injection of the fluorescent carbocyanine dye Dil in goldfish and image acquisition using a fluorescence microscope equipped with rhodamine optics [21]. In rodents, in-vivo imaging of RGCs can be much more readily achieved by using transgenic mice that express CFP under the control of a Thy-1 promoter construct, and modified cSLO [22]. In primates, RGCs have been studied in vivo by labelling the cells with retrograde transport of rhodamine dextran injected into the lateral geniculate nucleus [23]. Fluorescence images of these ganglion cells were obtained by means of adaptive optics SLO. The scan field size was restricted to 1° or 2° in order to concentrate the argon--krypton laser tuned to 530 nm on a chosen area.

Axons and dendritic arborization could be highly resolved in primate RGCs in vivo, comparing favourably in detail with ex-vivo confocal images of the same cells (Fig. 2). Hence, this in-vivo imaging method visualized structures smaller than the somas of typical retinal cells in living eyes [23].

In the field of cell-based therapies for ocular disease, two-colour imaging by confocal scanning laser microscopy can be used to localize transplanted cells. Bone-marrow-derived, lineage-negative haematopoietic GFP-expressing stem cells injected into the vitreous cavity of mice have been shown to target the retinal vasculature after 2 weeks. Using two-colour in-vivo imaging, visualization of cells and ocular vessels was possible simultaneously. This permitted verification of cell targeting and tracking of the fate of these cells in the living animal after experimental manipulation (Fig. 3) [24].

### **Ultrasound**

Ultrasound imaging employs acoustic transducers that send and receive ultrasound frequency energy in order to generate three-dimensional images from either reflection or through-transmission. The portability and relatively low cost of ultrasound technology render it ideal for in-vivo imaging both in animals and in human patients. In ophthalmology, 10-, 20-, 40- and 50-MHz ultrasound probes are in use. However, resolution is still the limiting factor. Recent advances in imaging technology and the development of targeted microbubble contrast agents hold promise for molecular imaging in the future [25,26].

### **Nuclear imaging**

Radionuclide-based imaging includes positron emission tomography (PET) and single photon emission computed tomography (SPECT), in which internal radiation is administered through an imaging probe labelled with a radioisotope. These imaging probes can be labelled peptides, proteins or antibodies. The major advantages of radionuclide-based molecular imaging are high sensitivity, quantifiability and lack of tissue penetration limit. Attenuation of the central visual pathway due to unilateral hypertension glaucoma was studied in monkeys using PET. In 2-[<sup>18</sup>F]fluoro-2-deoxy-glucose studies, monocular visual stimulation of the eye with elevated intraocular pressure yielded significantly reduced neural responses in the occipital visuocortical areas. The response reduction was limited to the visual cortex ipsilateral to the affected eye, indicating the unique vulnerability of ipsilateral visual cortex in experimental unilateral glaucoma. In addition, in [<sup>11</sup>C]PK11195 PET studies selective accumulation of activated microglia, a sign of neural degeneration, was found in lateral geniculate nuclei in both hemispheres [27].

### **Computed tomography**

Computed tomography (CT) is among the leading imaging modalities in clinical radiology. Although the low sensitivity of CT precludes its use beyond morphologic imaging, CT offers several advantages over other imaging techniques. First, it provides excellent spatial anatomic resolution in the sub-millimetre range. Second, it is a fast modality allowing acquisition of whole-body images within a few seconds. Third, it is widely available in Western countries. Although stand-alone CT does not play a significant role in molecular imaging, it can complement PET by providing high-resolution anatomic information [28].

### **Magnetic resonance imaging**

Magnetic resonance imaging (MRI) has emerged as a powerful diagnostic tool providing non-invasive anatomical, physiological and functional information in a single examination. Although the spatial resolution of MRI is low compared with that of optical imaging techniques, MRI is independent of media opacities, unhampered by depth limitations and offers a large field of view. In clinical use, MRI provides better information about the optic nerve and the eye in the context of the orbit than any other ocular imaging modality. Recent advances have enabled its use for visualizing anatomy, physiology and function in the retina with laminar-specific resolution [29]. These techniques can be divided into high-resolution anatomical [T1, T2, diffusion-weighted MRI, gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) contrast MRI], physiological [blood-oxygenation-level-dependent (BOLD), blood-volume, blood-flow, and manganese-enhanced MRI (ME-MRI)], and functional imaging technologies.

Prognostic MRI biomarkers of treatment efficacy for retinopathy have been established, such as (A) preretinal vitreous oxygen level (a steady-state biomarker of *inner retinal oxygen tension*); (B) change in preretinal vitreous oxygen tension during a hyperoxic provocation (a functional biomarker of *vascular autoregulation*); (C) leakage of contrast agent into the vitreous [a steady-state biomarker of *blood–retinal barrier (BRB) permeability surface area product*]; and (D) retinal uptake of systemically administered manganese  $Mn^{2+}$  during a visual task (a functional biomarker of *intraretinal ion regulation*) [30]. These four areas are now described in more detail.

(A) The retina is one of the most metabolically active tissues in the body and has a very high oxygen demand. Concentrations have been determined by  $^{19}F$ -MRI in rats

with experimental retinopathy of prematurity, reflecting preretinal vitreous oxygen tension [31]. Preretinal vitreous oxygen has also been measured by  $^{19}\text{F}$  magnetic resonance spectroscopy using a perfluorocarbon droplet in rats that had been experimentally diabetic for 9–13 months [32].

(B) Retinal vessel autoregulation is the major pathophysiology involved in diabetic retinopathy and other retinal vascular disease. Functional MRI (fMRI) was used to quantitatively and non-invasively assess the preretinal vitreous oxygenation response to a hyperoxic provocation ( $\Delta\text{PO}_2$ ) [33]. It was shown that abnormal  $\Delta\text{PO}_2$  could be measured before the appearance of retinal lesions.  $\Delta\text{PO}_2$  as measured with fMRI can therefore be a clinically relevant prognostic biomarker of the development of vascular pathology and drug treatment efficacy [33]. Alternative methods using the BOLD-MRI technique are being developed to provide information about the perfusion/oxygenation index, but these have yet to demonstrate their ability to distinguish between inner and outer retinal signal intensity changes associated with oxygenation [29,30].

(C) Dynamic contrast-enhanced MRI (DCE-MRI) using Gd-DTPA has proven very useful in studying the BRB in various conditions and retinopathy models. DCE-MRI can detect even small changes in vascular permeability. These quantifiable changes in BRB permeability have proven useful for the assessment of various therapeutic interventions, including corticosteroid treatment and anti-vascular endothelial growth factor therapy [30].

(D) Ion activity plays a central role in photoreceptor transduction, retinal neuronal transmitter release, regulation of gap junction conductance and modulation of postsynaptic potentials in RGCs. Ionic control is also important in the cell cycle and in neuronal development. Cellular ion demand has been used as an index of viability in proliferating cells [30]. High-resolution ME-MRI data allow accurate measurement of

the thickness of different retinal layers, which can be clearly distinguished from the choroid. After systemic injection of  $\text{MnCl}_2$ , paramagnetic manganese ( $\text{Mn}^{2+}$ ), an MRI contrast agent and ion analogue, readily accumulates intracellularly as a function of membrane integrity and cellular activation, and is removed relatively slowly (Fig. 4). Therefore, ME-MRI is ideally suited for determining how small changes in ion activity and retinal thickness are spatially and temporally linked to progression of retinopathy, ocular injury and glaucoma [29,30]. Other studies have investigated intravitreally administered  $\text{Mn}^{2+}$  to highlight the optic nerve and examine its transport [34].

MRI, however, can also serve to examine biomarkers in the anterior chamber:

Kolodny et al. used Gd-DTPA to examine the anterior protein pathway in rabbit eyes by contrast-enhanced MRI [35]. Dynamic studies of water movement in rat eyes were performed employing a deuterium MRI ( $^2\text{H}$ -MRI) analysis technique. The rat eye was imaged very selectively using a spin-echo pulse sequence after intraperitoneal injection of deuterated saline. Aqueous flow rates of 0.09–0.11/min in the rabbit and 0.4/min in the rat could be measured [36]. Water diffusion parallel or perpendicular to the axonal tracts of the optic nerve can be determined by diffusion MRI, which permits specific detection of axonal and myelin damage in mouse models of optic nerve injury [37].

One of the principal emerging applications of MRI molecular imaging is ocular drug delivery research. Possible contrast agents for drug-delivery imaging include small contrast ions ( $\text{Mn}^{2+}$ ,  $\text{Gd}^{3+}$ ), small chelated contrast ions (Mn and Gd complexes:  $\text{Mn-EDTA}^{2-}$ ,  $\text{Gd-DTPA}^{2-}$  and  $\text{Gd-DOTA}^{2-}$ ), iron oxide contrast agents (iron oxide particles: superparamagnetic iron oxide nanoparticles), contrast-labelled macromolecules (peptides, oligonucleotides, proteins, RNA, DNA), contrast-labelled synthetic polymers (polyanions, polycations and neutral polymers that resemble

macromolecules or drug carriers) and contrast-labelled drug-delivery systems (nanoparticles, microspheres, micelles, liposomes, polymeric carriers for drug delivery) [38]. Besides the above-mentioned indirect measurements of drug effects such as the effect of pharmaceuticals on BRB, direct measurement of contrast agents as drug surrogates will increase in the future. First MRI studies have been performed investigating the pathways of electric current in transcorneal and transscleral iontophoresis [39]. In another study, Kim et al. used MRI to investigate drug delivery to the posterior segment of the eye from a sustained-release implant placed on the episclera and in the vitreous cavity using a contrast agent. The concentration profile, clearance and pharmacokinetics of the contrast agent in the eye showed that drug elimination from the subconjunctival space into the conjunctival lymphatics created a significant trans-scleral barrier for intraocular delivery of the contrast agent, making episcleral implants hardly effective in delivering the drugs and contrast agent into the vitreous humour [40,41]. Others have examined the distribution and clearance of a macromolecule, Gd-labelled albumin, after subconjunctival injection by MRI [42]. Jockovich and co-workers recently used MRI to localize and monitor an anecortave acetate formulation administered periocularly to the eye using a posterior juxtасcleral injection technique [43].

## **IMAGING TOOLS AND TARGETS**

### **Reporter gene imaging and cell labelling**

Reporter imaging can use optical approaches, with reporter genes expressing optically detectable molecules such as GFP or CFP, as mentioned above. Optical approaches provide inexpensive, readily available methods for imaging reporter gene

expression and are widely used in current eye research. The pathophysiology of certain disease processes like inflammation or proliferation can be imaged optically by direct cell labelling with fluorescent dyes such as acridine orange, rhodamine G6, or carboxyfluorescein-diacetate-succinimidyl ester (CFSE). Details have been provided above. In contrast to cells labelled by gene expression, the direct labelling of cells permits analysis of cell proliferation, because the label is diluted after cellular division. This approach has become the standard technique for measuring immune cell proliferation [44] and has recently been introduced to eye research (Alex A, under review).

### **Antibody labelling**

Imaging using larger molecules such as monoclonal antibodies and fragments has been used frequently in tumour research in other medical fields. The range of probes (and therefore imaging modalities) is rather wide, and comprises optical, ultrasound, and MR-based probes. Preclinical studies, e.g. using specific antibodies conjugated to gadolinium or magnetic nanoparticles, demonstrated the feasibility of MRI antibody imaging in cells and animal models [25]. VEGFR imaging studies are already being performed in gastrointestinal cancer using  $^{123}\text{I}$ -VEGF165 [45]. Others have just developed a VEGFR-2 specific PET tracer [45]. Antibody labelling in benign proliferative eye disease such as age-related macular degeneration or diabetic retinopathy could be the optimal imaging modality for drug targeting in the future.

### **Protein expression (labelling of proteins, peptides, oligonucleotides)**

Several methods can be used to measure biosynthesis as an indicator of cell proliferation, with approaches targeted towards protein synthesis and membrane synthesis. The uptake of labelled amino acids, such as  $^{11}\text{C}$ -methionine, has been

shown to correlate with tumour growth [25]. Artificial amino acids have also been tested as indicators of amino acid transport [25]. These methods may provide new insight into physiological and pathophysiological pathways in the eye.

### **Enzyme activity**

The most popular enzyme-related approach has been based upon the use of viral thymidine kinase, combined with labelled probes that are specific substrates for phosphorylation by viral thymidine kinase [25]. Using this method, optical imaging of cell proliferation in proliferating eye disease may become possible in the future. Luciferase-based imaging, which has been described above, also falls into this category.

### **Nanoparticles**

Real-time measurements of biological/chemical/physical processes are an ultimate goal for in-vivo studies. Nanoscale devices are typically smaller than several hundred nanometres and are comparable in size to large biological molecules such as enzymes, receptors and antibodies [26]. About 100 to 10,000 times smaller than human cells, these nanoscale devices can offer unprecedented interactions with biomolecules both on the cell surface and inside the cells, which may revolutionize disease diagnostics and treatment. The best-studied nanomaterials include quantum dots, nanoshells, gold, liposomes, iron oxides, perfluorocarbon, nanotubes and dendrimers. Nanoshells and gold nanocages have been reported for optical coherence tomography in vitro [26]. With ongoing rapid advances in nanotechnology and instrumentation, these systems will soon be capable of sensing at the single-molecule level at any point of interest within the living cell. They will also be capable of simultaneously detecting multiple analytes and physical parameters.

## **Apoptosis**

Apoptosis imaging has been developed from an extension of annexin V staining in vitro, which indicates apoptotic cells through binding to phosphatidyl serines. The molecules are normally found only on the inner surface of plasma membranes and are therefore not accessible to annexin V. However, during apoptosis, these molecules are transiently exposed to the extracellular space, allowing binding of annexin. Whereas in other medical fields applications of annexin-based apoptosis imaging have been developed for SPECT ( $^{99m}\text{Tc}$ -annexin), PET, MRI and ultrasound, optical imaging has been established in ophthalmology, as described above.

## **SUMMARY AND FUTURE DIRECTIONS**

Molecular imaging represents a very powerful tool for translating basic science into clinical benefit. It offers a way to assess and quantify pathways in both animal models and patients. In addition, molecular imaging can be used in ocular drug-delivery research, from drug development and application pathways to pharmacodynamic measures for optimal dosing. This is especially important now that intravitreal pharmacologic treatments such as anti-VEGF or steroid therapy have become available. While optical imaging methods are already widely established in eye research due to the eye's unique optical properties, advances in other imaging modalities will provide insight into molecular pathways not directly visible by means of optical systems. With the advances in MRI technology and the emergence of more sophisticated MRI systems, this method will also continue to help scientists to gain insights into the mechanisms of ocular drug delivery and to develop and monitor

effective medication for eye disease.

## Figure legends

### Fig. 1:

Scanning laser ophthalmoscopic examination of CX<sub>3</sub>CR1-reporter mice at different time points following laser-induced injury. Left: red-free mode; right: fluorescence imaging with an excitation of 488 nm.

Taken from: Eter N. Invest Ophthalmol Vis Sci 2008;49: 3649-58

### Fig.2:

Comparison of in-vivo and ex-vivo images in a monkey. (a, d) In-vivo images from two retinal locations. (b, e) Ex-vivo images of the same locations made with a low-power (0.3-NA) objective to simulate the NA of the in-vivo conditions. (c, f) Ex-vivo images of the same locations made with a 0.85-NA objective to show details of the cell structure. In-vivo images were acquired with a 2° field of view yielding 256 pixels per degree. High-NA ex-vivo images are summed images from a focus stack including the ganglion cell layer and the inner plexiform layer. The nerve fibre layer was excluded to increase visibility of the dendrites. The in-vivo images are a registered average of 1000 raw video frames. Images have been rescaled and contrast enhanced for display purposes. Scale bars: 25 µm (a–c), 50 µm (d–f).

Taken from: Gray DC et al. Invest Ophthalmol Vis Sci 2008;49:467-473

### Fig.3:

Localization of intravitreally injected cells in vivo. GFP-expressing bone marrow-derived, lineage-negative haematopoietic stem cells were injected into the vitreous of mice that received intravenous infusion of red dye. (a) Injected cells (green)

concentrated near the optic disc area from which the hyaloid vessels (red) emerged.

(b) Two weeks after injection, GFP<sup>+</sup> cells persisted in the vitreous and, in some cases, targeted and adhered to segments of the regressing hyaloid vasculature.

Scale bar: 200  $\mu$ m.

Taken from: Ritter et al., Invest Ophthalmol Vis Sci. 2005;46:3021-3026

Fig.4:

Left: High-resolution ME-MRI (23.4 mm axial resolution) of a rat eye in vivo.

Identification of post-receptor retina (Post-Rec.). Inset: Agreement in whole retinal (WR) and post-receptor (Post-rec.) thickness between ME-MRI and thin slice histology (Hist). GC: ganglion cell layer; IP: inner plexiform layer; IN: inner nuclear layer; ON: outer nuclear layer; PR: photoreceptor layer. Error bars show the SEM, and the numbers above the bars are the numbers of animals studied.

Taken from: Berkowitz BA & Roberts R. NMR Biomed.2008;21:957-967

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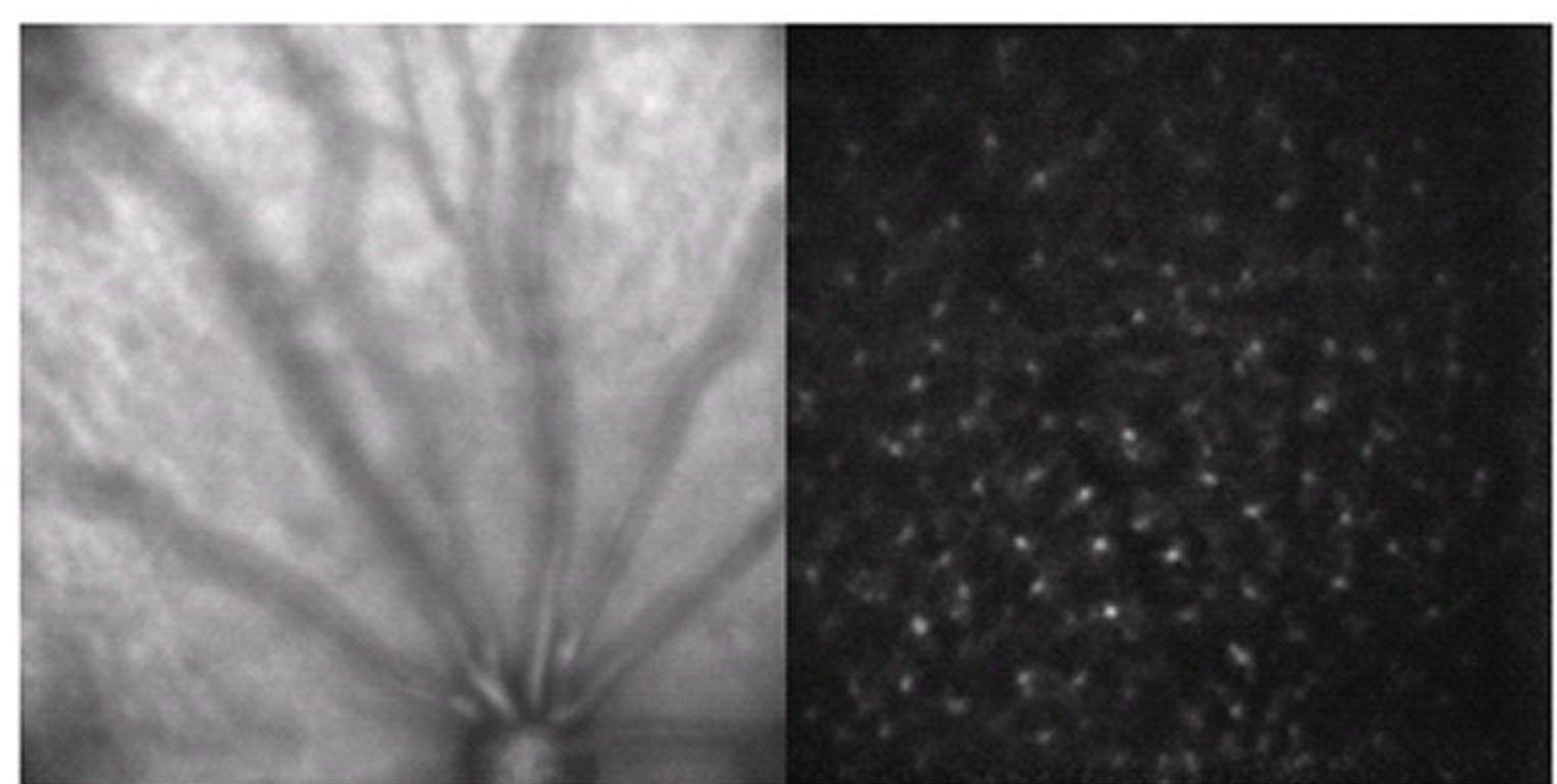
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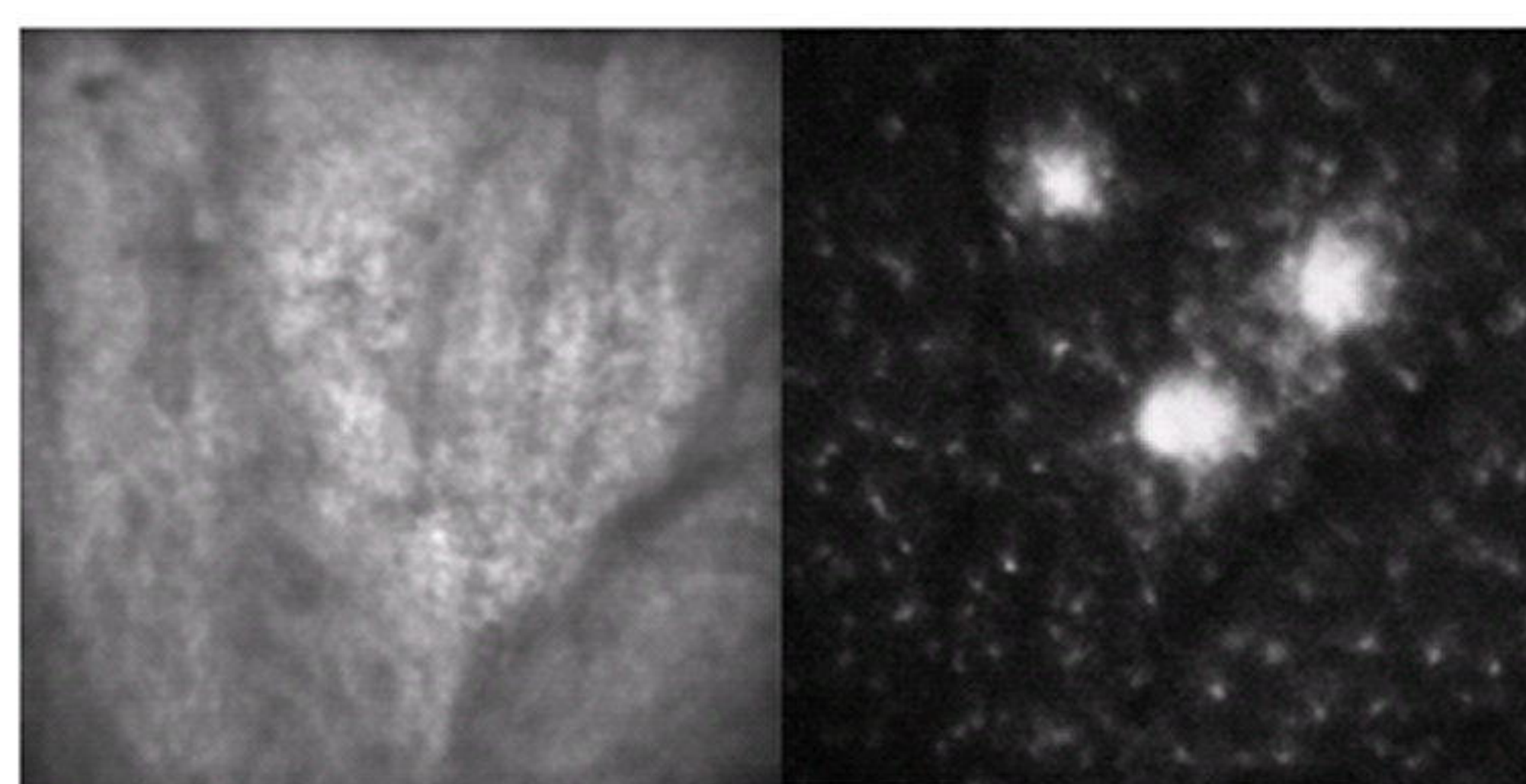
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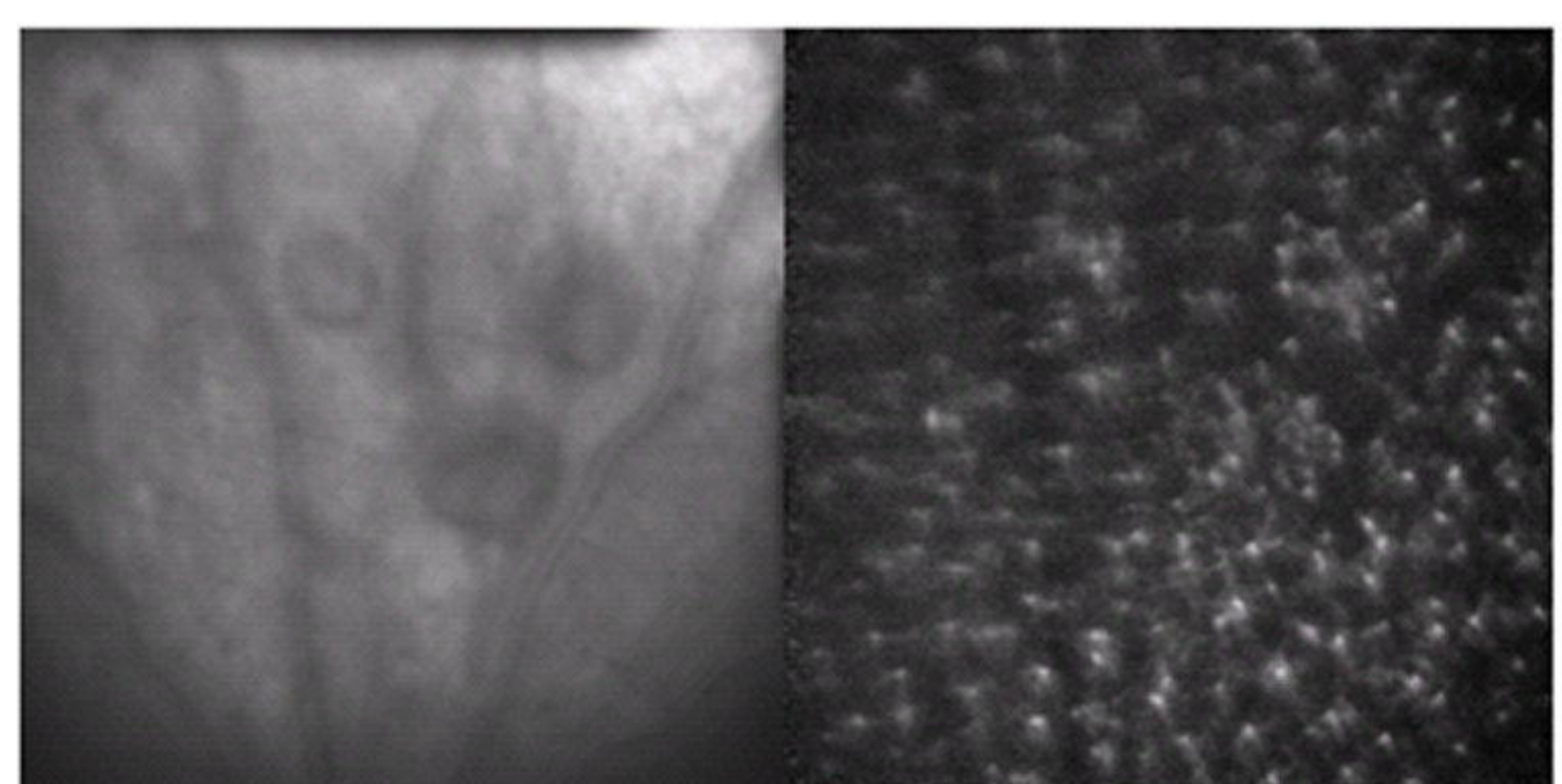
Figure 1



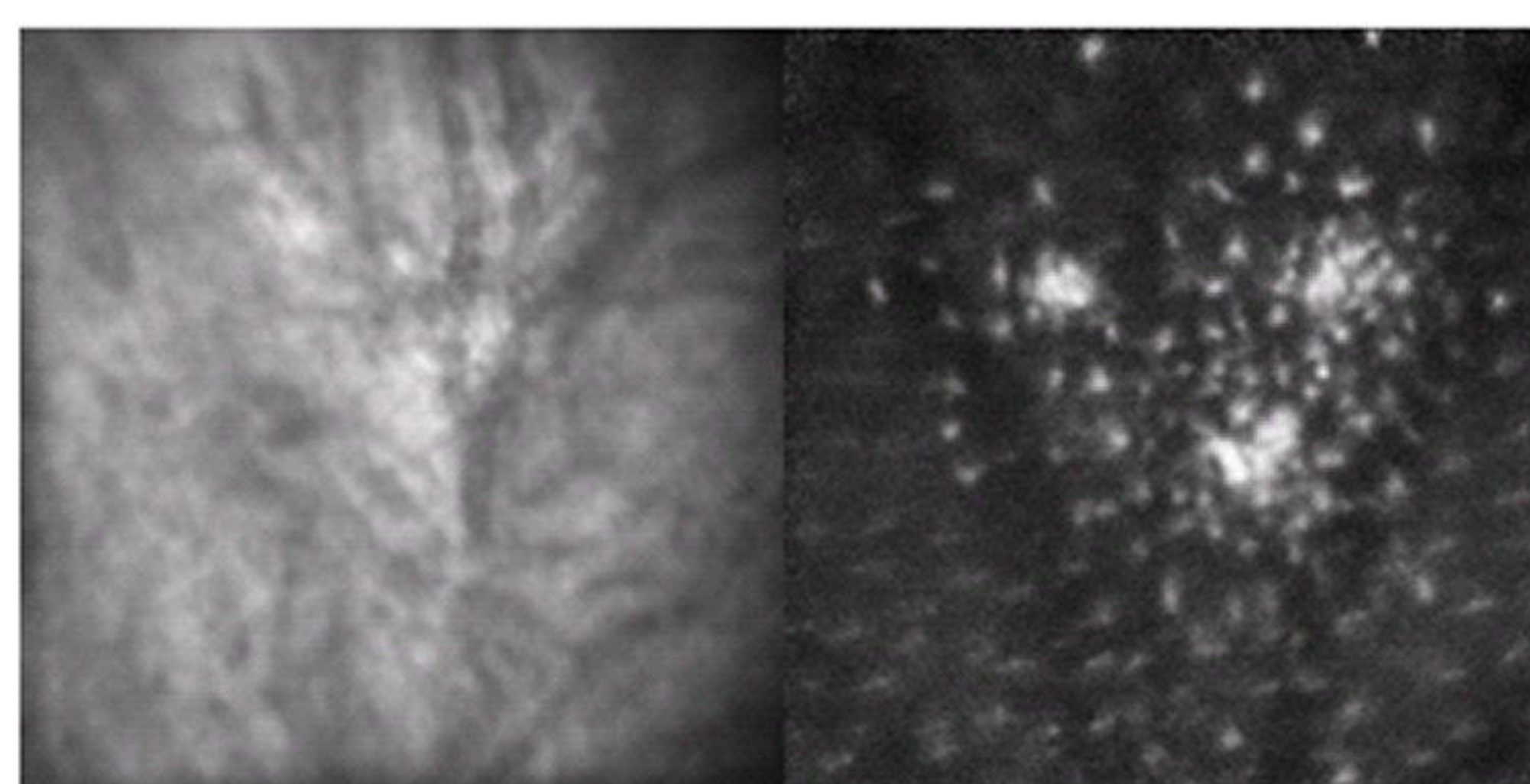
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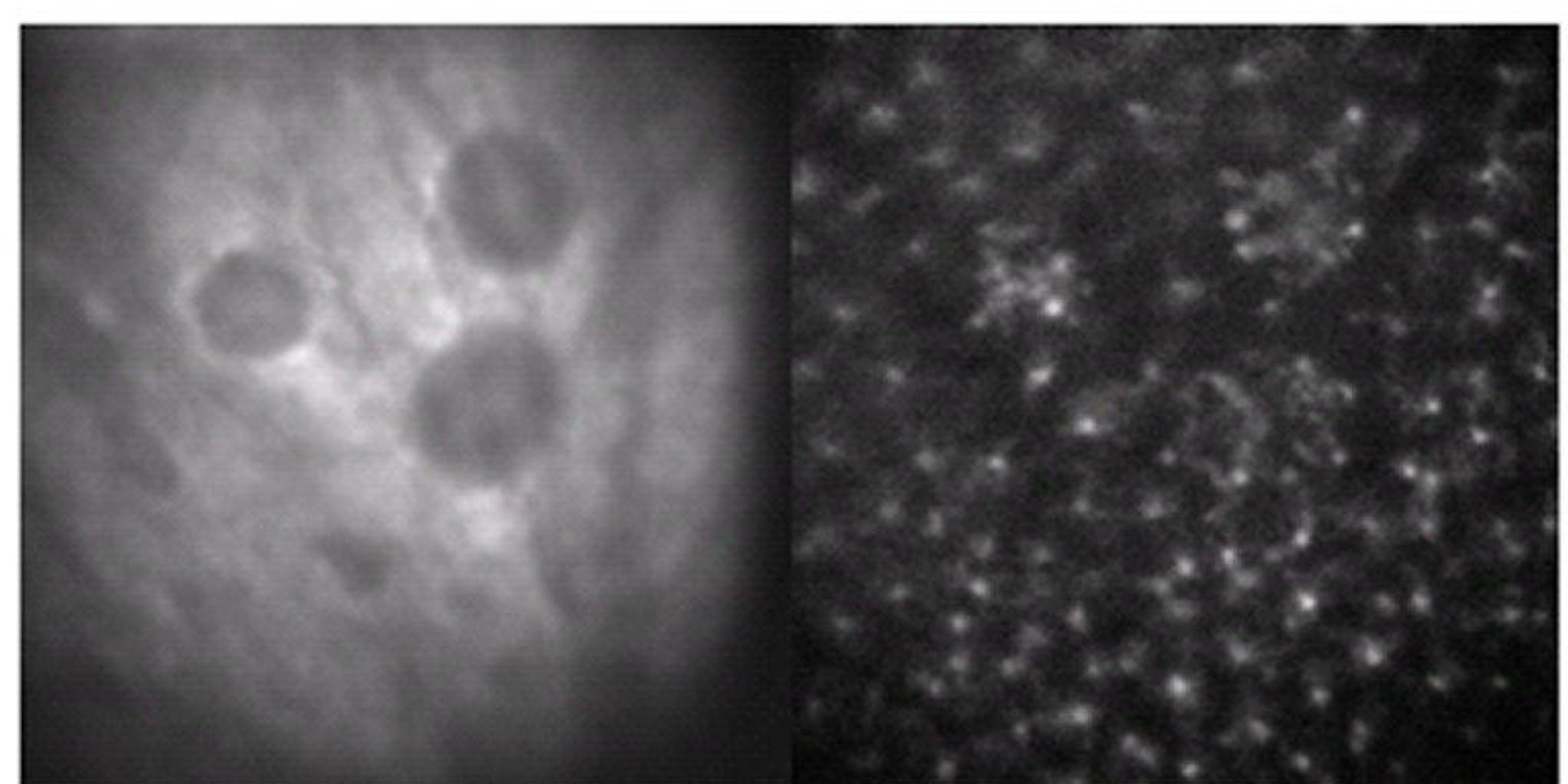
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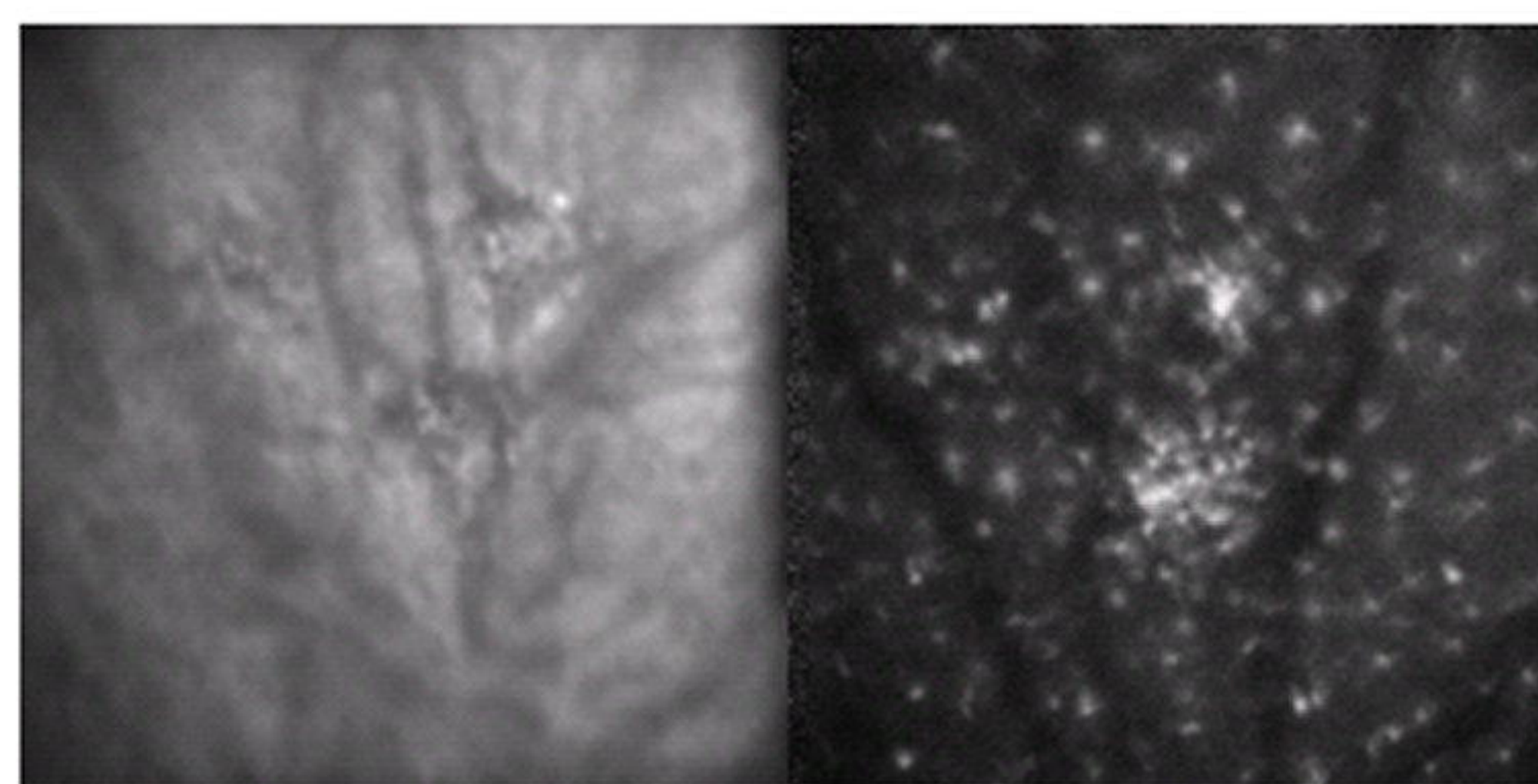
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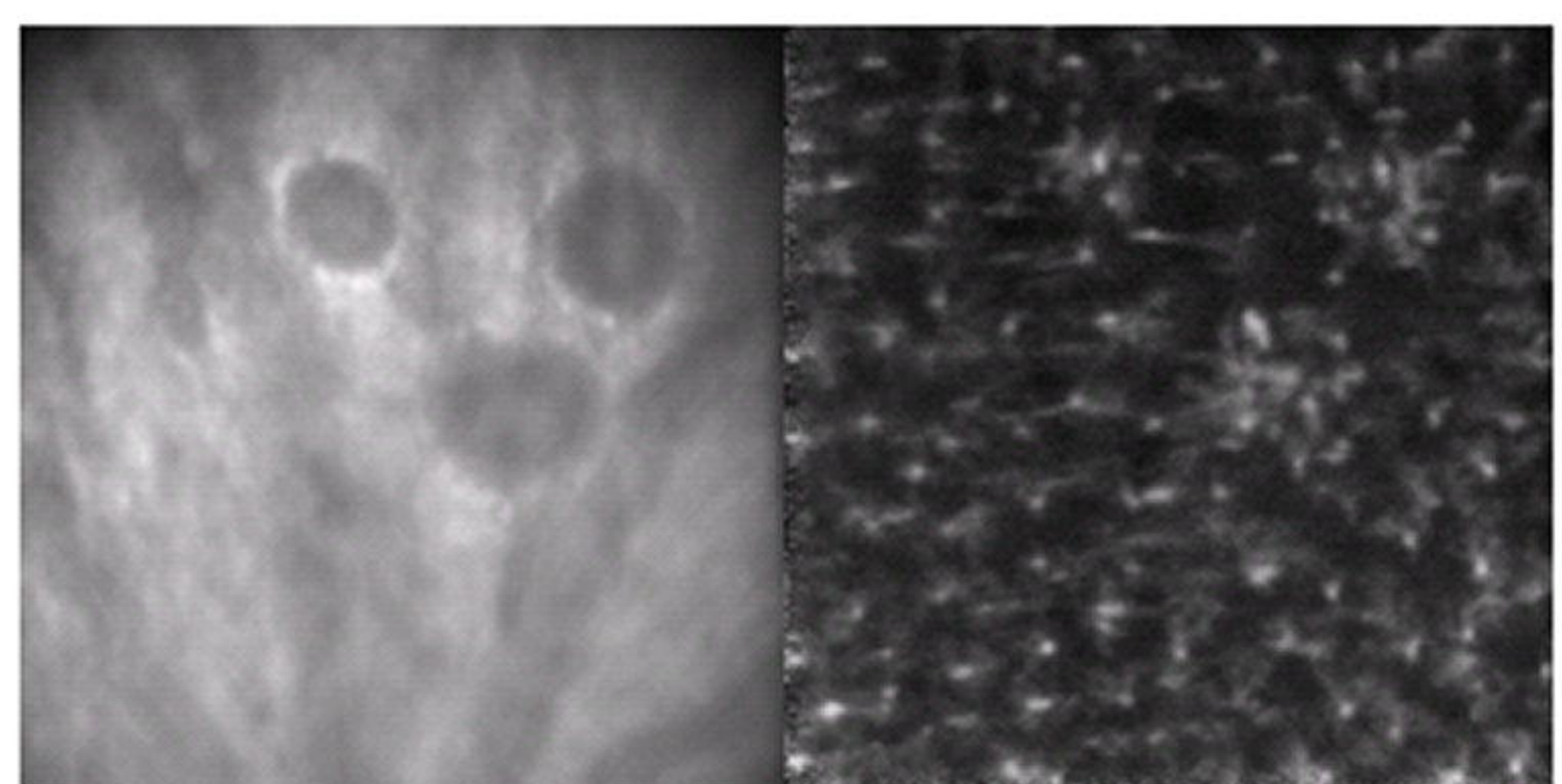
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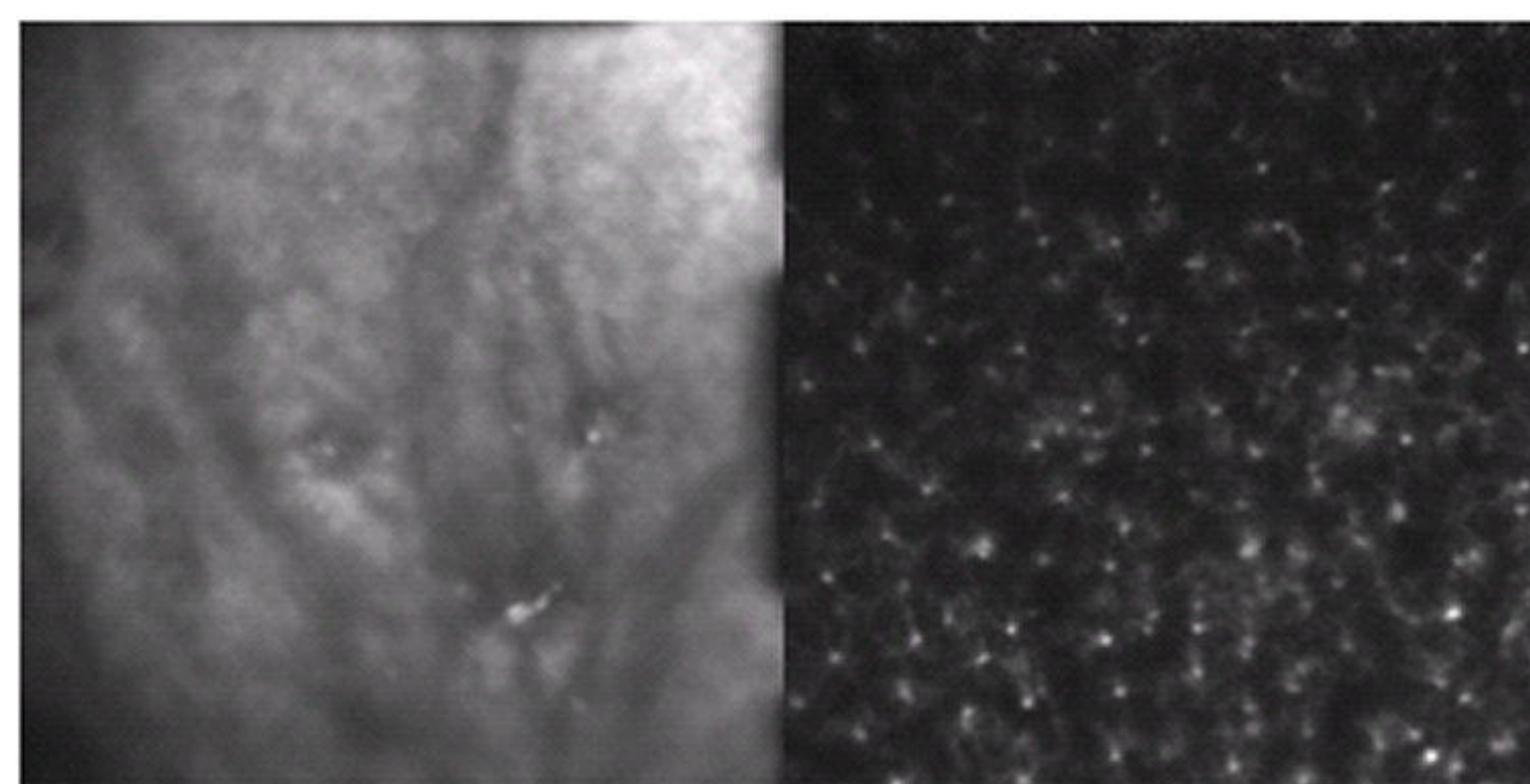
2 h



2 w

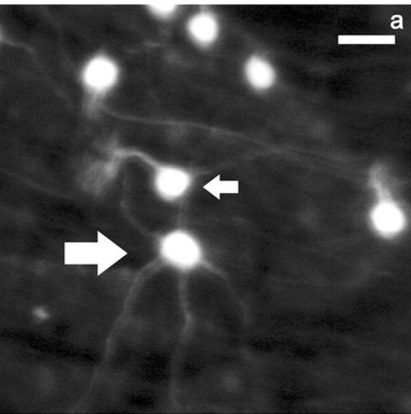


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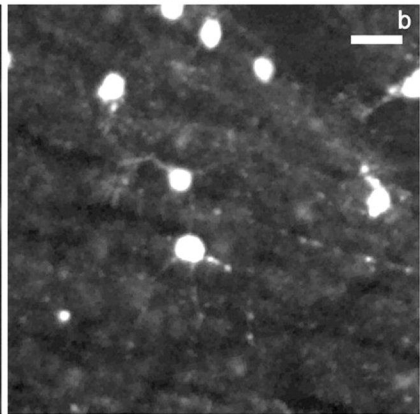


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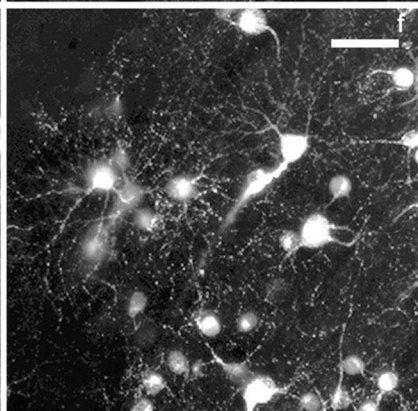
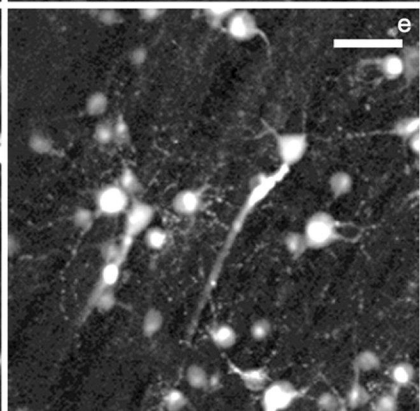
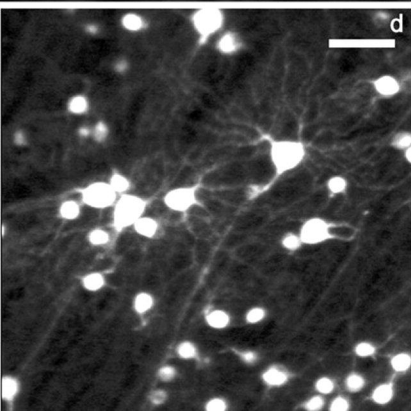
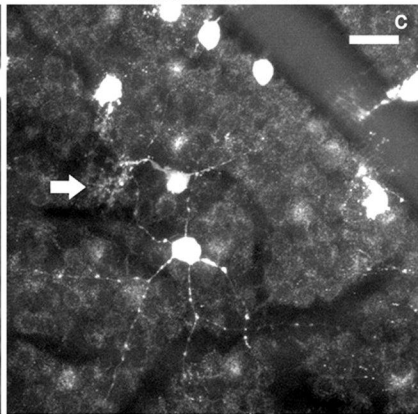
*in vivo* 0.25 NA



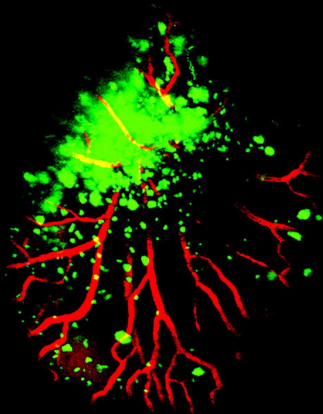
*ex vivo* 0.3 NA



*ex vivo* 0.85 NA



a



b

