**University of
Zurich**^{UZH}

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2020

Cortex-wide microcirculation mapping with ultrafast large-field multifocal illumination microscopy

Chen, Zhenyue ; Zhou, Quanyu ; Rebling, Johannes ; Razansky, Daniel

Abstract: The recently introduced large-field multifocal illumination (LMI) fluorescence microscopy technique opened new possibilities for transcranial observations of mouse brain dynamics with a unique combination of capillary level resolution and centimeter-scale field-of-view (FOV). Here we report on a new acceleration scheme for LMI based on raster scan of a lattice pattern combined with a parallel camera exposure scheme, which attains 200 Hz frame rate over 12×12 mm² FOV with 7.5 μ m spatial resolution. We demonstrate real-time transcranial in vivo tracking of particles and imaging of microcirculation across the entire mouse cortex, thus corroborating the superb spatiotemporal resolution performance of LMI unattainable with other techniques. Potential applications include investigations into cerebrovascular function, cell tracking, as well as large-scale functional neuroimaging.

DOI: <https://doi.org/10.1002/jbio.202000198>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-198513>

Journal Article

Accepted Version

Originally published at:

Chen, Zhenyue; Zhou, Quanyu; Rebling, Johannes; Razansky, Daniel (2020). Cortex-wide microcirculation mapping with ultrafast large-field multifocal illumination microscopy. *Journal of Biophotonics*, 13(11):e202000198.

DOI: <https://doi.org/10.1002/jbio.202000198>

AUTHOR QUERY FORM

Dear Author,

During the preparation of your manuscript for publication, the questions listed below have arisen. Please attend to these matters and return this form with your proof.

Many thanks for your assistance.

Query References	Query	Remarks
Q1	Please confirm that given names (blue) and surnames/family names (vermilion) have been identified and spelled correctly.	
Q2	Please check if link to ORCID is correct.	
Q3	Please provide expansion for the term “PIV,” if applicable.	
Q4	Please provide the “volume number” for reference 3.	

Funding Info Query Form

Please confirm that the funding sponsor list below was correctly extracted from your article: that it includes all funders and that the text has been matched to the correct FundRef Registry organization names. If no FundRef Registry organization name has been identified, it may be that the funder was not found in the FundRef registry, or there are multiple funders matched in the FundRef registry. If a name was not found in the FundRef registry, it may not be the canonical name form, it may be a program name rather than an organization name, or it may be an organization not yet included in FundRef Registry. If you know of another name form or a parent organization name for a “not found” item on this list below, please share that information.

Funding Agency	FundRef Organization Name
H2020 European Research Council	H2020 European Research Council;
H2020 Marie Skłodowska-Curie Actions	H2020 Marie Skłodowska-Curie Actions

FULL ARTICLE

Cortex-wide microcirculation mapping with ultrafast large-field multifocal illumination microscopy

Zhenyue Chen | Quanyu Zhou | Johannes Rebling | Daniel Razansky*

Institute for Biomedical Engineering and
Institute of Pharmacology and Toxicology,
University of Zurich and ETH Zurich,
Zurich, Switzerland

*Correspondence

Daniel Razansky, Institute for Biomedical
Engineering, Wolfgang-Pauli-Str. 27, 8093
Zurich, Switzerland.

Email: daniel.razansky@uzh.ch

Funding information

H2020 European Research Council,
Grant/Award Number: ERC-2015-CoG-
682379; H2020 Marie Skłodowska-Curie
Actions, Grant/Award Number: 746430

Abstract

The recently introduced large-field multifocal illumination (LMI) fluorescence microscopy technique opened new possibilities for transcranial observations of mouse brain dynamics with a unique combination of capillary level resolution and centimeter-scale field-of-view (FOV). Here we report on a new acceleration scheme for LMI based on raster scan of a lattice pattern combined with a parallel camera exposure scheme, which attains 200 Hz frame rate over $12 \times 12 \text{ mm}^2$ FOV with $7.5 \mu\text{m}$ spatial resolution. We demonstrate real-time transcranial in vivo tracking of particles and imaging of microcirculation across the entire mouse cortex, thus corroborating the superb spatiotemporal resolution performance of LMI unattainable with other techniques. Potential applications include investigations into cerebrovascular function, cell tracking, as well as large-scale functional neuroimaging.

KEYWORDS

brain dynamics, cerebral vasculature, fluorescence microscopy, in vivo tracking, microcirculation, ultrafast imaging

1 | INTRODUCTION

Fluorescence microscopy has proven as a highly versatile tool for biological discovery, unveiling structural and functional features on cellular and sub-cellular scales. Widefield microscopy exhibits low-cost and high-speed but impeded by diminished contrast and spatial resolution when imaging through highly scattering living

tissues. Conventional laser-scanning microscopy (LSM) methods, including confocal and multiphoton microscopy [1–3], are able to approach diffraction-limited spatial resolution. However, their frame rate is intrinsically limited by the scanning speed of galvanometers, microelectromechanical systems or acousto-optic deflector (AOD) devices, as well as by the integration time of optical detectors (e.g. photomultiplier tubes or photodiodes) and fluorescent label efficiency. These challenges prohibit the use of LSM for visualizing rapid biological

processes occurring on the millisecond scale, such as blood perfusion or fast neuronal responses.

One strategy of balancing between spatial resolution and imaging speed consists in employing parallel excitation and/or acquisition schemes, such as light-field [4], light-sheet [5] or structured illumination microscopy [6]. These emerging techniques have increased the frame rate to unprecedented levels as compared with traditional LSM yet remained applicable to imaging of relatively shallow depth or transparent samples. Multifocal illumination microscopy schemes, for example, spinning-disk confocal microscopy [7], are aimed at improving frame rates without compromising spatial resolution and imaging depth. For example, multifocal structured illumination microscopy with a digital micromirror device has enabled dynamic imaging of multicellular organisms at 1 Hz [8]. Two-photon microscopy with a microlens array has recently achieved a frame rate of up to 1 kHz and was applied to in vivo recordings of Ca^{2+} transients [9]. Yet, fast imaging comes at the detriment of restricted (sub-millimeter) FOV combined with a micrometer-scale depth of field. The sample can be additionally scanned with a mechanical stage thus rendering larger FOV by stitching multiple individual scans, which, however, may take minutes to hours to accomplish. Finally, optical-resolution optoacoustic microscopy has recently demonstrated label-free imaging of murine cerebrovascular dynamics with high resolution [10]. Yet, imaging of large FOVs implied the use of mechanical transducer scanning, which resulted in effective acquisition times in the minutes range, for example, when imaging the entire mouse cortex [11, 12].

Herein, we report on a parallel exposure (PE) scheme for fast large-field multifocal illumination (LMI) fluorescence microscopy capable of accelerating its effective frame rate to 200 frame-per-second (fps), a 20-fold improvement over the originally reported implementation [13], without sacrificing spatial resolution and depth of focus or the imaged FOV. A combination of a two-dimensional AOD and a beam-splitting grating was employed to generate and scan a multifocal lattice-pattern illumination synchronized with a high-speed camera for fluorescence signal detection. The core of the proposed PE-LMI approach is to scan the multi-foci lattice multiple times within one camera exposure cycle. Different from the conventional raster scanning of a single spot/pattern during each exposure cycle of the camera, the parallel exposure regime allows scanning a lattice-pattern multiple times (e.g. 3×3) within a single camera exposure window thus boost the effective frame rate.

2 | MATERIALS AND METHODS

2.1 | The PE-LMI setup

The system layout is depicted in Figure 1A. The laser beam from a continuous (CW) laser is expanded to fill the aperture of an AOD (DTSXY-400-532, AA Opto-Electronic, France), which provides a scanning frequency up to 95 kHz with maximum diffraction efficiency at 15-bit scanning precision for a beam diameter of 7.5 mm. The deflected laser beam is guided to a custom-made beam-splitting grating (Holoeye Photonics AG, Germany) to generate 21×21 beamlets with 74% diffraction efficiency. The angle of adjacent diffraction order of beam-splitting grating is 0.57° , scanned through AOD to cover the imaged FOV of $12 \times 12 \text{ mm}^2$. The beamlets are projected to the sample plane with a scan lens (CLS-SL, EFL = 70 mm, Thorlabs, NJ) to generate the “lattice-pattern” illumination shown in Figure 1C. The imaging part works in reflection mode as the emitted fluorescence signal first passes through the dichroic mirror and then gets focused onto the sensor plane of the camera (pco.dimax S1, PCO AG, Germany) with a camera lens (AF micro-Nikkor 60 mm, Nikon, Japan). The high-speed camera features a maximum 4.467 kHz frame rate at full resolution of 1008×1008 pixels. To enable the PE scanning strategy, the camera was operated in an external control mode with its trigger signal synchronized with a fixed AOD scanning pattern (e.g. 3×3), as illustrated in Figure 1B,C. A digital I/O card (NI-PCIe 6535b, National Instrument, USA) was employed to output trigger signals to the AOD for the rapid beam steering. A custom-made programmable, microcontroller-based triggering unit was used to synchronize the AOD and camera.

2.2 | Data processing

Image reconstruction was performed with a custom algorithm written in MATLAB (R2019b, MathWorks, MA). The reconstruction procedure includes three steps: (a) localization of illumination pattern, (b) digital pinholing, and (c) signal superimposition. First, for each scanning frame, local maxima corresponding to the illumination spots are identified through adaptive threshold combined with connected-component analysis. Note that image interpolation should be performed before the first step in the case of over-sampling. In the second step, local maxima were extracted from each scanning frame and filtered with a digital pinhole to suppress the crosstalk from the adjacent illumination spots and

FIGURE 1 System setup and characterization. A Schematic of the developed PE-LMI system. B, Trigger scheme of AOD and camera under the PE mode. C, Scanning pattern and direction in one 3×3 scanning block captured by a single camera snapshot. Solid disks represent the lattice pattern resulting from one pattern captured by a single camera exposure while hollow disks are the additional lattice patterns in the PE mode. D Spot size in the horizontal and vertical direction with fitted Gaussian function on the focal plane. E, Change of spot size in the horizontal and vertical direction along z axis. F, Integrated intensity of individual beamlets in the lattice-pattern normalized to the mean value (5.44% SD from mean value). G and H, Comparison of lateral resolution performance between widefield and PE-LMI images in the same ROI. I, Spatial resolution characterization by Gaussian fitting of the line profile along the green line in G and H. AOD, acousto-optic deflector; PE-LMI, parallel exposure large-field multifocal illumination; ROI, regions of interest

out-of-focus fluorescence. The digital pinhole was defined as a 2D Gaussian mask with the SD which was adjusted according to the magnification ratio, scanning steps and scattering properties of the sample. For each sub-frame, the sub-regions centered at the excitation spot are extracted and multiplied with pre-defined Gaussian mask to form the filtered image. When compared with conventional widefield imaging, LMI image with the virtual pinholing implementation maintains high lateral

resolution across highly-scattering samples (Figure S1 and S2). Finally, the filtered sub-frames are superimposed to render the final compounded high-resolution image.

2.3 | System characterization

For basic characterization of the PE-LMI system, we employed 473 nm CW laser (FPYL-473-1000-LED,

Frankfurt Laser company, Germany). A beam profiler (SP928, Ophir Optonics, USA) was used to record the lattice-pattern on the focal plane. The spot size in the horizontal and vertical directions of the illumination pattern was determined by the full-width-at-half-maximum (FWHM) after Gaussian fitting. To measure the uniformity error of the lattice-pattern, the intensity of each individual beamlet was calculated through integrating the intensity around its peak value with a radius calculated at $1/e^2$ of the peak value. In addition, the change of spot size along z axis was measured with a motorized stage using 200 μm steps.

The lateral resolution was characterized with orange-yellow fluorescent beads (460/594 nm, 1–5 μm diameter, Cospheric, USA). The beads were mixed with distilled water, spread onto a standard microscope slide and further fixed with a coverslip. The sample was scanned with $\sim 3 \mu\text{m}$ scanning step. The lateral resolution was empirically determined by the FWHM of Gaussian-fitted point-spread function (PSF).

2.4 | In vivo mouse imaging

To showcase the accelerated imaging speed of PE-LMI, we explored the feasibility of tracking fluorescent beads and recording fluorescent dye perfusion across the entire cerebrovascular network. For in vivo particle tracking, an Athymic nude-Fox1nu mouse (2 month old, Harlan Laboratories LTD, Switzerland) was anesthetized with isoflurane (4.0% vol/vol for induction and 1.5%–2.0% vol/vol for maintenance) containing mixed air and oxygen at flow rates of 0.8 L/min and 0.2 L/min, respectively. To reduce light scattering, the scalp was removed after subcutaneous injection of 1.4 μL analgesics (10 mg/mL lidocaine and 10 $\mu\text{g/mL}$ epinephrine). Fluorescent beads (460/594 nm, 1–5 μm diameter, Cospheric, USA) were administrated intravenously through the tail vein. An effective frame rate of 80 Hz was attained by employing 15×15 scanning positions, 18 kHz beam scanning speed, 3×3 scans per single camera exposure, and 2 kHz camera acquisition rate.

Similarly, for dynamic cerebrovascular mapping, one Athymic nude-Fox1nu mouse (2 month old, Harlan Laboratories LTD, Switzerland) was used. After anesthesia and scalp removal as described above, 100 μL cy5.5 (1 mg/mL, Invitrogen, USA) was administered through the tail vein. An effective frame rate of 200 Hz was achieved with 15×15 scanning positions at 45 kHz beam scanning speed using parallel exposure to 3×3 scan blocks. Mice were euthanized after the experiment while still under anesthesia. All animal experiments were

performed in accordance with the Swiss Federal Act on Animal Protection and approved by the Cantonal Veterinary Office Zurich.

3 | RESULTS

3.1 | Spatio-temporal resolution performance

The FWHM spot size in the horizontal and vertical direction was 14.41 μm and 11.00 μm , as evaluated from Figure 1D. The depth of focus is $\sim 1 \text{ mm}$ along z-axis within spot size of 15 μm (Figure 1E). Figure 1F shows that the intensity uniformity error of individual beamlets is around $\pm 5.44\%$. After image reconstruction, the lateral resolution was characterized by measuring the FWHM of PSF in both the widefield image (Figure 1G) and corresponding PE-LMI image (Figure 1H), resulting in 17.25 μm spatial resolution for widefield image and 7.50 μm for LMI (Figure 1I). This approximately corresponds to half the size of the illumination spot, which is in agreement with the theory on spatial resolution improvement of image scanning microscopy [14].

The imaging speed of PE-LMI hangs upon a number of factors, including the camera acquisition speed, brightness of the fluorescent agent, AOD scanning speed, and number of scanning positions required to form the compounded image. For instance, if 15×15 scanning positions are required to reconstruct one PE-LMI image, 200 fps is achievable with 45 kHz scanning rate. For scanning the illumination pattern 3×3 times within one camera exposure cycle, 5 kHz data acquisition rate of the camera is required. Overall, 15×15 scanning positions were sufficient to arrive at high-quality in vivo images of the mouse brain.

3.2 | In vivo particle tracking across the entire mouse cortex

The intravenously injected fluorescent beads were tracked simultaneously across the entire mouse cortex (Figure 2A). In two regions of interest (ROIs), we further depicted the trajectories of bead flow with color-encoded velocities (Figure 2B) with PIV code [15]. Two representative trajectories of fluorescent beads are illustrated in Figure 2C. The calculated velocity of the first bead was $\sim 1.73 \text{ mm/s}$ while the other one was flowing at a much faster speed of $\sim 4.27 \text{ mm/s}$. Since each PE-LMI image is reconstructed at an interval of 12.5 ms, the displacement of the second bead was roughly 53.4 μm within one

FIGURE 2 In vivo tracking of fluorescent beads. A, Whole-cortex cerebrovascular mapping through localization of fluorescent beads over 16.5 seconds total recording time. B), Local velocity mapping of ROIs indicated by the brown and purple squares in A. C, Trajectories of two fluorescent beads in ROIs indicated by the blue and green boxes in A. Red arrows indicate the “tailing” events. ROI, regions of interest

image frame, resulting in “tailing” phenomenon while no apparent image artifacts were recognized at lower flow velocities.

3.3 | Dynamic cerebral microcirculation mapping

Figure 3A displays the time-lapse images at certain time points during the perfusion process recorder over 10 second. It is observed that the anterior cerebral artery (ACA) and middle cerebral artery (MCA) are pronounced within 2 second post dye injection while superior sagittal sinus are pronounced at ~3 second post-injection, which is in agreement with the literature [16]. A map of the perfusion constant across the entire mouse brain was then generated via time-to-peak analysis (color-coded in Figure 3B), in which both the structural information and functional information is displayed. Two ROIs close to the ACA and MCA regions are chosen to display the millisecond-scale signal variation. The time-lapse

PE-LMI images from these two ROIs are shown in Figure 3C,D, respectively. The branching effect of the vascular tree can be clearly discerned by closely observing the fluorescence signal increase, in particular when color-coding the vessel perfusion process according to the signal onset time (Figure 3E,F).

4 | DISCUSSION

Our proposed method has a unique combination of centimeter-scale FOV, high spatial resolution and imaging speed. Owing to the sparse excitation introduced by the illumination grid and digital pinholing implementation in the image reconstruction, superior image contrast can be achieved due to the fact that photon scattering is significantly mitigated. Compared to conventional LSM achieving MHz pixel rate with sequential scanning strategy, our PE-LMI system is capable of approaching GHz pixel rates. To further accelerate the imaging speed while maintaining the same FOV and resolution, one may

FIGURE 3 Dynamic cerebral microcirculation mapping with dye perfusion. A, Transcranial imaging of cerebrovascular perfusion across entire mouse cortex recorded at 200 Hz frame rate. Data acquired with 15×15 scanning positions at 45 kHz scanning rate and 3×3 scans per single camera exposure. B, Time-to-peak map. C and D, Time-lapse images of ROIs close to ACA and MCA recorded at 200 Hz frame rate. White circles indicate the newly appearing vessels. E and F, Vessel branching process becomes apparent when the perfusion process is color coded according to the onset time of the fluorescence signal. Similar to the time-to-peak analysis, an onset threshold of 0.2 was applied to the normalized images in C and D. ACA, anterior cerebral artery; MCA, middle cerebral artery; SSS, superior sagittal sinus; ROI, regions of interest

think of increasing the pattern density. This can readily be achieved by integrating more scans into a single camera exposure cycle, or by simply applying a higher-order diffraction beam-splitting grating. However, higher pattern density may result in cross talk between neighboring foci when imaging scattering samples. The multifocal illumination is converted into uniform illumination when density of the foci reaches infinity. Thus, the optimal pattern density depends on scattering properties of the imaged sample. Compared to high diffraction order gratings, our proposed scanning scheme has significant advantages since: (a) it provides flexibility for adjusting the scanning pattern depending on the scattering properties of the sample, for example, 5×5 or more scans per single camera exposure can be selected for thin or transparent samples, while 3×3 scans are better tailored for in vivo imaging of strongly scattering mammalian brains, as shown in this work; (b) design and fabrication of high-

order diffraction gratings (e.g. with 101×101 orders) with small uniformity errors $<10\%$ is nontrivial let alone the fact that a submicron critical dimension will impose significant challenges on the fabrication process; (c) it requires much higher output power of the laser to maintain sufficient levels of light intensity at the sample surface with a high-order diffraction beamsplitting grating. In fact, the choice of affordable high-power laser sources with output power exceeding 500 mW is limited to very selected wavelengths. Eventually, the imaging speed will be limited by the noise equivalent fluorescence signal or the signal-to-noise ratio of the imaging system.

5 | CONCLUSION

In summary, we proposed an ultrafast large-field fluorescence microscopy method based on a beamsplitting

grating and two-dimensional AOD scanner together with a parallel camera exposure scheme. It can be readily integrated into commercial microscopes, thus establishing an effective bridge between widefield macroscopy and LSM. The PE-LMI method significantly improves upon imaging performance of the conventional widefield fluorescence imaging techniques by attaining superior image contrast and maintaining high spatial resolution by mitigating the effects of scattering in living tissues. It thus opens new prospects for studying fast biodynamics, such as cerebral microcirculation, tracking of fluorescently labeled stem cells or circulating tumor cells, or large-scale functional neuroimaging.

ACKNOWLEDGMENTS

The authors would like to thank M. Reiss for the assistance in animal experimentation. This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie Grant Agreement No. 746430 - MSIOAM and the European Research Council Consolidator Grant ERC-2015-CoG-682379.

CONFLICTS OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Zhenyue Chen conceived the concept. Quanyu Zhou and Zhenyue Chen performed the experiments and data analysis. Johannes Rebling designed and fabricated the trigger unit. Daniel Razansky supervised the project. All authors discussed the results and contributed to writing the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Quanyu Zhou <https://orcid.org/0000-0001-7719-4649>

REFERENCES

- [1] S. Hell, E. H. K. Stelzer, *J. Opt. Soc. Am. A* **1992**, 9, 2159.
- [2] W. Denk, J. H. Strickler, W. W. Webb, *Science* **1990**, 248, 73.
- [3] N. G. Horton, K. Wang, D. Kobat, C. G. Clark, F. W. Wise, C. B. Schaffer, C. Xu, *Nat Photonics* **2013**, 7.
- [4] R. Prevedel, Y.-G. Yoon, M. Hoffmann, N. Pak, G. Wetzstein, S. Kato, T. Schrödel, R. Raskar, M. Zimmer, E. S. Boyden, *Nat. Methods* **2014**, 11, 727.
- [5] P. J. Keller, A. D. Schmidt, J. Wittbrodt, *Science* **2008**, 322, 1065.
- [6] M. G. Gustafsson, *J. Microsc.* **2000**, 198, 82.
- [7] T. Tanaami, S. Otsuki, N. Tomosada, Y. Kosugi, M. Shimizu, H. Ishida, *Appl. Opt.* **2002**, 41, 4704.
- [8] A. G. York, S. H. Parekh, D. Dalle Nogare, R. S. Fischer, K. Temprine, M. Mione, A. B. Chitnis, C. A. Combs, H. Shroff, *Nat. Methods* **2012**, 9, 749.
- [9] T. Zhang, O. Hernandez, R. Chrapkiewicz, A. Shai, M. J. Wagner, Y. Zhang, C.-H. Wu, J. Z. Li, M. Inoue, Y. Gong, *Nat. Methods* **2019**, 16, 1119.
- [10] H. Estrada, J. Turner, M. Kneipp, D. Razansky, *Laser Phys. Lett.* **2014**, 11, 045601.
- [11] J. Rebling, H. Estrada, S. Gottschalk, G. Sela, M. Zwack, G. Wissmeyer, V. Ntziachristos, D. Razansky, *J. Biophotonics* **2018**, 11, e201800057.
- [12] H. Estrada, J. Rebling, W. Sievert, D. Hladik, U. Hofmann, S. Gottschalk, S. Tapio, G. Multhoff, D. Razansky, *Bone* **2020**, 133, 115251.
- [13] Z. Chen, B. Mc Larney, J. Rebling, X. L. Deán-Ben, Q. Zhou, S. Gottschalk, D. Razansky, *Laser Photonics Rev.* **2020**, 14, 1900070.
- [14] C. B. Müller, J. Enderlein, *Phys. Rev. Lett.* **2010**, 104, 198101.
- [15] O. T. Bruns, T. S. Bischof, D. K. Harris, D. Franke, Y. Shi, L. Riedemann, A. Bartelt, F. B. Jaworski, J. A. Carr, C. J. Rowlands, *Nat. Biomed. Eng.* **2017**, 1, 1.
- [16] V. Kalchenko, D. Israeli, Y. Kuznetsov, A. Harmelin, *Sci. Rep.* **2014**, 4, 5839.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Chen Z, Zhou Q, Rebling J, Razansky D. Cortex-wide microcirculation mapping with ultrafast large-field multifocal illumination microscopy. *J. Biophotonics*. 2020;e202000198. <https://doi.org/10.1002/jbio.202000198>

Graphical Abstract

The contents of this page will be used as part of the graphical abstract of html only.
It will not be published as part of main.

Conventional laser scanning microscopy is subject to hard tradeoffs between resolution, field of view (FOV) and imaging speed. We report on a parallel exposure scheme for large-field multifocal illumination (LMI) fluorescence microscopy. The new technique can visualize centimeter-scale FOVs with capillary level resolution and 200 Hz frame rate, thus opening new venues for studying fast biodynamics, such as cerebral microcirculation, circulating cells or brain signaling.