Computational Miniature Mesoscope V2: A deep learning-augmented miniaturized microscope for single-shot 3D high-resolution fluorescence imaging

Deep learning-augmented Computational Miniature Mesoscope

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Abstract

Computational Miniature Mesoscope (CM²) is a recently developed computational imaging system that enables single-shot 3D imaging across a wide field-of-view (FOV) using a compact optical platform. In this work, we present CM² V2 – an advanced CM² system that integrates novel hardware improvements and a new deep learning reconstruction algorithm. The platform features a 3D-printed freeform LED collimator that achieves ~80% excitation efficiency – a ~3X improvement over our V1 design, and a hybrid emission filter design that improves the measurement contrast by >5X. The new computational pipeline includes an accurate and computationally efficient 3D linear shift-variant (LSV) forward model and a novel multi-module CM²Net deep learning model. As compared to the model-based deconvolution in our V1 system, CM²Net achieves ~8X better axial localization and ~1400X faster reconstruction speed. In addition, CM²Net consistently achieves high detection performance and superior axial localization across a wide FOV at a variety of conditions. Trained entirely on our 3D-LSV simulator generated training data set, CM²Net generalizes well to real experiments. We experimentally demonstrate that CM²Net achieves accurate 3D reconstruction of fluorescent emitters across a ~7-mm FOV and 800-µm depth, and provides ~7-µm lateral and ~25-µm axial resolution. We anticipate that this simple and low-cost computational miniature imaging system will be impactful to many large-scale 3D fluorescence imaging applications.
1 Introduction

Fluorescence microscopy techniques have made tremendous progress and become essential to image biological structures and dynamics\textsuperscript{1,2}. However, there are still two major challenges to address to fulfill the \textit{unmet need} of providing cellular resolution across a cortex-wide field-of-view (FOV) in freely behaving animals. This first challenge is to overcome the barrier of \textit{imaging at large scale while preserving resolution}\textsuperscript{3}. Recently developed \textit{table-top} systems, such as RUSH\textsuperscript{4} and COSMOS\textsuperscript{5}, are only beginning to enable multiscale measurements with sufficient spatial resolution. However, they are still confounded by scale-dependent optical aberrations\textsuperscript{6}, which results in undesirable tradeoffs between the FOV, resolution, and system complexity. The second challenge is to perform \textit{mesoscale} imaging of thousands of neurons spanning multiple brain regions during naturalistic behaviors \textit{using a head-mounted “wearable” device}. Recent progress in miniaturized head-mounted fluorescence microscopes, \textit{i.e.}, miniscopes\textsuperscript{7,8}, has enabled unprecedented views of activity in the neural circuits underlying diverse behaviors. However, the imaging capability of current miniscopes remains restricted by their optics. Most of the current miniscopes rely on a gradient index (GRIN) objective lens\textsuperscript{7}, which limits the FOVs to \(<1 \text{ mm}^2\), confining measurements to a subset of cells within a single brain region. Wide-FOV miniscopes have recently been developed by replacing the GRIN with a compound lens, such as WideScope\textsuperscript{9}, cScope\textsuperscript{10}, mini-mScope\textsuperscript{11}, Kiloscope\textsuperscript{12}, and Miniscope-LFOV\textsuperscript{13}, but only at the cost of degraded resolution and increased size, weight and, system complexity. In general, fundamental physical limits preclude meeting the joint requirements of FOV, resolution, and miniaturization using conventional optics.

Computational imaging techniques have shown unique capabilities of overcoming the limitations of conventional optics by jointly designing optics and algorithms. Lightfield microscopy (LFM) and related technologies, such as integral microscopy\textsuperscript{14} and Fourier LFM\textsuperscript{15}, achieve single-shot high-resolution 3D fluorescence imaging\textsuperscript{16–20}. LFM works by attaching a microlens array (MLA) to an existing microscope to jointly collect spatial and angular information, which enables reconstructing the 3D fluorescence from a single shot. While miniaturized LFM, such as MiniLFM\textsuperscript{21} and miniscope3D\textsuperscript{22}, enabled single-shot 3D fluorescence imaging using modified miniscope platforms, its FOV is fundamentally limited by the GRIN lens. Lensless imaging is another computational imaging technique for single-shot 3D imaging, where a mask\textsuperscript{23,24} or a diffuser (a random microlens array)\textsuperscript{25,26} is placed directly in front of a CMOS, such as FlatScope\textsuperscript{23,27}, DiffuserScope\textsuperscript{25}, and GEOMScope\textsuperscript{26}. However, the removal of focusing optics imposes penalties to the measurement’s contrast and signal-to-noise ratio (SNR)\textsuperscript{3}, severely limiting their sensitivity for imaging weak fluorescent signals\textsuperscript{5}.

We recently developed Computational Miniature Mesoscope (CM\textsuperscript{2})\textsuperscript{28} that aims to overcome all the key limitations of FOV, resolution, contrast, SNR, and size and weight in existing miniature fluorescence imaging systems. Notably, the CM\textsuperscript{2} combines the best of both LFM and lensless designs. It places a \(3 \times 3\) MLA directly in front of a CMOS sensor for imaging, like the lensless design. This ensures compactness and light weight while further exploiting the microlens’s focusing power for providing high image contrast. The CM\textsuperscript{2} captures an image with multiple viewpoints (\textit{i.e.}, parallax), which enables robust recovery of 3D fluorescence in a single shot, like the LFM. In our previous work, we demonstrated the CM\textsuperscript{2} V1 system that achieved single-shot 3D fluorescence imaging across \(7 \times 8 \text{ mm}^2\) FOVs with \(\sim 7\)-\(\mu\text{m}\) lateral resolution and \(\sim 200\)-\(\mu\text{m}\) axial resolution\textsuperscript{28}. Notably, the CM\textsuperscript{2} V1 was the first \textit{standalone} computational miniature fluorescence microscope with an integrated illumination module on the same platform. Using a four-LED array arranged in an oblique epi-illumination geometry, the CM\textsuperscript{2} V1 can uniformly illuminate a \(\sim 1\)-\(\text{cm}^2\) FOV and
achieved a ~24% overall light efficiency. In this work, we significantly advance the CM² technology. We report the CM² V2 system that integrates innovations in both hardware and computation to address several limitations in light efficiency, image contrast, and reconstruction quality and speed, as summarized in Fig. 1.

Figure 1: Overview of the Computational Miniature Mesoscope (CM²) V2. (a) The CM² V2 hardware platform features freeform LED illuminators for high-efficiency excitation, hybrid filters for spectral leakage rejection, and a BSI CMOS sensor for high-SNR measurement. (b) A photo of the assembled CM² V2 prototype. (c) An example CM² measurement on a volume consisting of 10-µm fluorescent beads. (d) The CM²Net combines view demixing (demix), view synthesis (synthesis) and lightfield refocusing (RF) enhancement (enhance) modules to achieve high-resolution, fast, and artifact-free 3D reconstruction. (e) The CM²Net reconstruction from the measurement in (c), spanning a 7-mm FOV of 800-µm depth range.

On the hardware side, we present several updates in the V2 platform that significantly improves the light throughput and image contrast. First, the oblique LED illuminator in the CM² V1 was inefficient due to the lack of collimation optics. To address this issue while preserving the compactness and light weight of the design, the CM² V2 platform integrates a miniature LED collimator designed based on freeform optics²⁹,³⁰ (Fig. 1a). Freeform optics is well suited for realizing miniature optics due to its design flexibility, compactness, and high optical performance. We present a rapid-prototyping process for fabricating such miniature optics using clear resin on a table-top 3D printer. Each 3D-printed LED collimator weighs only ~0.03 grams. The upgraded four-LED array illuminator achieves ~80% efficiency, a ~3x improvement over the V1 design, and provides a highly confined, uniform illumination with up to 75 mW excitation power across an 8-mm diameter circular region. Second, the CM² V2 integrates a hybrid emission filter³¹ that combines an interference filter and an absorption filter (Fig. 1a), which significantly suppresses the spectral leakage suffered by the CM² V1. Built around a back-side illuminated (BSI) CMOS sensor (Fig. 1a, b), the CM² V2 achieves an overall 5x improvement in the image contrast and captures high-SNR measurements in a variety of experimental conditions, as shown in our experiments (an example raw image shown in Fig. 1c).

On the computation side, we present a deep learning model to achieve high-quality 3D recovery across a wide FOV with significantly improved axial resolution and reconstruction speed. In recent years, deep learning has emerged as the state-of-the-art for solving many ill-posed inverse problems in imaging³². Directly related to this work, deep learning techniques for single-shot 3D fluorescent reconstruction have been developed for standard widefield³³ and LFM³⁴–³⁷ measurements. Deep learning-based super-resolution reconstruction has been achieved on various fluorescence microscopy techniques³⁸–⁴⁰. To
devise a robust and accurate deep learning model, we consider several key characteristics in the image formation of the CM\textsuperscript{2}. Notably, the measurement contains 3 \times 3 overlapped views and lightfield information, and the exact forward model is 3D linear shift variant (LSV)\textsuperscript{28}. Our deep learning model, termed CM\textsuperscript{2}Net, solves the highly ill-posed single-shot 3D reconstruction problem using three functional modules (Fig. 1d). The “view demixing” module separates the single measurement into 3 \times 3 non-overlapping views by exploiting the distinct aberrated image features from the view-dependent point spread functions (PSF). The “view-synthesis” module and the “lightfield refocusing enhancement” module jointly perform high-resolution 3D reconstruction by utilizing the lightfield information.

To incorporate the 3D-LSV information into the deep learning model, we develop an accurate and efficient 3D-LSV forward model for synthesizing CM\textsuperscript{2} measurements from 3D distributed fluorescent emitters. Our 3D-LSV model is based on a low-rank approximation using a small number of experimentally calibrated PSFs taken on a sparse 3D grid. A key difference between our 3D-LSV model and a recently developed depth-wise LSV model\textsuperscript{22,34} is that we reduce the model complexity by a global decomposition for all the PSFs and represent them with a shared set of basis PSFs. In addition, we show that the added axial interpolation capability in our 3D model achieves “axial super-resolution” beyond the large axial step used in the PSF calibration. We generate all the training data using this 3D-LSV simulator to train CM\textsuperscript{2}Net, which bypasses the need for physically acquiring a large-scale training data set in experiments.

We first quantitatively evaluate the CM\textsuperscript{2}Net’s performance for reconstructing 3D distributed fluorescent emitters in simulation. Our results demonstrate that the trained CM\textsuperscript{2}Net can consistently provide high-quality 3D reconstructions and is robust to variations in the imaging FOV and fluorescent emitter’s size, intensity, 3D location, and seeding density. Our ablation studies highlight that the view-demixing module significantly reduces the false positive rates in the reconstruction and the joint view-synthesis and lightfield refocusing enhancement framework provides highly accurate 3D reconstructions. We quantify the CM\textsuperscript{2}Net’s detection performance on a testing set containing fluorescent emitters with the seeding density and SNR approximately matching in-vivo cortex-wide one-photon Calcium imaging\textsuperscript{5} across \textasciitilde7 mm FOVs. CM\textsuperscript{2}Net achieves averaged recall and precision of 0.7 and 0.94 respectively, which are comparable to the state-of-the-art deep learning-based neuron detection pipeline\textsuperscript{41}.

In addition, we show that the 3D-LSV simulator-trained CM\textsuperscript{2}Net generalizes well to real experiments and provides high-quality 3D reconstructions (an example reconstruction on 10-µm fluorescent beads shown in Fig. 1e). We further demonstrate the trained model’s robustness to variations in emitter’s local contrast and SNR on samples containing mixed 10-µm and 15-µm fluorescent beads. Notably, CM\textsuperscript{2}Net enhances the axial resolution to \textasciitilde24-µm on experimental data, which is \textasciitilde8 \times better than the model-based reconstruction. The 3D reconstructions are validated against table-top widefield measurements across the CM\textsuperscript{2}’s \textasciitilde7-mm FOV and \textasciitilde0.8 mm imaging depth range. The reconstruction quality is quantitatively evaluated using recall, precision, and F1-score, and show nearly uniform detection performance across the whole FOV with few mis-detections and false positives. These results highlight that the 3D-LSV simulator-trained CM\textsuperscript{2}Net can accurately and robustly handle the strong shift variance across the large imaging volume supported by the CM\textsuperscript{2} and provide high-quality 3D reconstructions. In addition, CM\textsuperscript{2}Net reduces the reconstruction time to \textasciitilde4 seconds for a volume spanning a 7-mm FOV and 0.8-mm depth using a standard 8 GB GPU, which is \textasciitilde1400 \times faster speed and \textasciitilde19 \times less memory cost than the V1 model-based algorithm.
Overall, our contribution is a novel deep learning-augmented miniaturized microscope that achieves single-shot 3D fluorescence imaging. We demonstrate the unique 3D high-resolution mesoscopic imaging capability of CM$^2$ V2 on fluorescent emitters with different sizes and seeding densities. Built on off-the-shelf and 3D-printed components, we expect this simple and low-cost miniature imaging system can be adopted in many biological and neuroscience research labs in the future. It may find utility in a wide range of large-scale 3D fluorescence imaging and neural recording applications.

## 2 Results

In this section, we first provide an overview of the CM$^2$ V2 hardware platform. Next, we describe the 3D-LSV model and the CM$^2$Net deep learning framework. Finally, we present simulation results and experimental validations.

### 2.1 The CM$^2$ V2 hardware platform

The CM$^2$ V2 is a standalone miniature fluorescence microscope that is built with off-the-shelf and 3D-printed optical components and a 3D-printed housing, as illustrated in Figs. 1a and 2a. It mainly consists of two parts, including a newly designed illumination module and an upgraded imaging module. As compared to the V1 platform, the V2 platform features freeform LED-collimators that improve the illumination efficiency by $\sim 3\times$, a hybrid emission filter design that improves the image contrast by $\sim 5\times$. In this section, we highlight a few insights in our new design and refer the additional details to Section 4.1 and Supplementary Information Sections S1 and S2.

![Figure 2: CM$^2$ V2 hardware platform.](image)

(a) A cross-sectional view of the CM$^2$ V2 platform. The platform incorporates an MLA for imaging, freeform illuminators, a hybrid interference-absorption emission filter pair, and a BSI CMOS sensor. (b) The freeform LED collimator combines a singlet and a TIR parabolic surface. (c) Zemax simulation of the four-LED array demonstrates high-efficiency, uniform excitation onto a confined 8-mm circular region. (d) Experimental validation of the illumination module. (e) The hybrid emission filter pair improves the raw measurement’s SBR by $> 5\times$ (sample: 10-μm fluorescent beads in clear resin). Intensity profiles taken from several fluorescent beads show the SBR improvement by the hybrid filter design.

To design the illumination module, our goal is to provide $\sim 0.5 \text{ mW/mm}^2$ excitation power at the sample surface, a typical requirement for one-photon Calcium imaging in mouse brains$^5$. Further considering our goal of covering a cortex-wide centimeter-scale...
FOV, the total excitation power required is \(~50\) mW. In addition, the illumination module needs to be highly efficient, so that the LED driving current is sufficiently low without incurring excessive heat burden. The latter is particularly important for wearable miniature optical devices since they are particularly prone to heat dissipation issues.

Our solution is to incorporate a compact, lightweight freeform collimator to each LED illuminator. We place the collimator in-between the surface-mounted LED and the excitation filter. The collimator drastically improves the excitation efficiency by two mechanisms, including providing light concentration and ensuring high transmittance through the interference-excitation filter. The collimator is based on a hybrid refraction-reflection freeform illuminator design\(^{30}\), as shown in Fig. 2b. It consists of two parts, including an inner refractive lenslet and an outer parabolic reflective surface. The lenslet collimates the light emitted by the LED within a \(~52\) degrees conical angle. The parabolic surface is designed to satisfy the total internal reflection (TIR) condition and collimates the light emitted at high angles. The LED is placed around the shared focal point of the lenslet and the parabolic refractor. Each freeform collimator is \(~4 \times 4 \times 1\) mm\(^3\) in size, weighs only \(~0.03\) grams, and is 3D-printed with clear resin. The design is first validated in Zemax and achieves an efficiency of \(~80\)%, which incorporates the confounding factors due to the finite-sized LED emitter, the broadband LED emission spectrum, and the angle-dependent transmission spectrum of the excitation filter.

Like the \(\text{CM}^2\) V1, the entire illumination module consists of an array of four LED illuminators that are placed symmetrically around the imaging module. After performing optimization in Zemax, the LED illuminator is placed \(~6.7\) mm away from the optical axis of the imaging module and tilted by \(~45\) degrees to direct the excitation light towards the center of the imaging FOV. Our Zemax simulation shows that this design provides nearly uniform illumination confined in an 8-mm circle, as shown in Fig. 2c. Our experimental validation on the fully assembled \(\text{CM}^2\) V2 platform on a green fluorescence calibration slide closely matches with the simulation, as shown in Fig. 2d. The total excitation power in the 8-mm region is measured to be up-to \(~75\) mW at \(~470\) nm excitation wavelength, making our design applicable to head-mounted Calcium imaging applications.

The imaging module is built around the same off-the-shelf \(3 \times 3\) MLA as the \(\text{CM}^2\) V1 platform to form a finite-conjugate imaging geometry with \(~0.57\) magnification. The main improvement is the incorporation of a hybrid interference-absorption emission filter pair to improve the signal-to-background ratio (SBR) in the raw measurement. An interference-emission filter is placed in front of the MLA. Since the light rays emitted from the object space at this plane have smaller divergence angles, this results in less leakage as compared to placing the filter after the MLA (in the V1 design) due to imperfect operation conditions. An additional long-pass absorption filter is placed after the MLA to further suppress the leakage light. The emission spectra of the emission and absorption filters are optimized for the green fluorescence, as detailed in Fig. S2. The SBR improvement by this hybrid filter design is quantified experimentally, as shown in Fig. 2e. As compared to the interference-filter only measurement, the hybrid filter improves the SBR by \(~5\)× on a phantom consisting of 10-\(\mu\)m fluorescence beads. This improvement makes the new \(\text{CM}^2\) V2 platform significantly more robust in low-light fluorescent imaging conditions.

The \(\text{CM}^2\) V2 platform is built around a backside-illuminated (BSI) CMOS sensor. The dome-shaped 3D-printed housing provides mechanical support of all the optical components and shielding from ambient light. The size and weight of the \(\text{CM}^2\) V2 is only limited by the availability of miniature CMOS sensors. The \(\text{CM}^2\) V2 prototype is \(~36 \times 36\)
× 15 mm³ in size, including the commercial CMOS PCB board. The custom parts excluding the PCB is ~20 × 20 × 13 mm³ in size and weighs only ~2.5 grams. Combined with the high light efficiency and image contrast, the CM² V2 is an attractive solution for large-scale 3D fluorescence imaging in a miniaturized platform.

2.2 3D Linear Shift Variant model of the CM²

Our goal is to build an accurate and efficient 3D linear shift variant (LSV) model to describe the CM² image formation. Using the synthetic data simulated from this model, we will later train the proposed CM²Net to perform 3D reconstruction. In this section, we describe a sparse PSF calibration procedure, a low-rank approximation-based 3D-LSV model, and the forward model to generate synthetic CM² measurement. Additional details to Sections 4.2 and 4.3 and Supplementary Information Section S3.

First, to calibrate the spatially varying PSFs, we scan a 5-µm point source on a 3-axis translation stage. The point source is scanned across an 8 mm × 8 mm × 1 mm volume with steps of 1 mm laterally and 100 µm axially, which yields a stack of 9 × 9 × 11 calibrated PSFs, as illustrated in Fig 3a (more details in Section 4.2). Several example calibrated PSFs are shown in Fig. 3b, which highlight the following key characteristics of the CM² image formation. At a given lateral position, the off-axis foci shift laterally with the depth, akin to the lightfield. At a given depth, the PSFs are still shift variant due to two main factors, including the spatially varying aberrations from the microlenses and the missing side foci at large off-axis locations (when lateral location > 1.7 mm) truncated by the limited CMOS sensor size and the pixel’s limited angular response. As a result, to fully characterize the CM² 3D PSF, it necessitates a 3D-LSV forward model. Unfortunately, scanning the point source on the entire dense grid at our desired 3D resolution (4.15 µm × 4.15 µm × 10 µm) across the targeted imaging volume (~8 mm × 8 mm × 1 mm) would require ~370 million PSF measurements, which is highly impractical. Next, we describe a computational procedure to address this challenge.

Next, we develop an accurate and computationally efficient 3D-LSV model with a sparse calibration scheme for simulating the CM² measurements. Our model is based on a low-rank approximation and further adapts it to the unique characteristics in the CM², in the following steps (more details in Sections 4.3 and S3).

1) We denote the sparsely calibrated PSFs as \( H(u, v; x, y, z) \), where \( (u, v) \) are the pixel coordinates of the PSF image, and \( (x, y, z) \) is the 3D location of the point source. In total, this calibrated PSF dataset contains \( N = 891 \) images. The native size of each PSF image contains ~6.4M pixels, which is too large to be directly operated on for the low-rank decomposition. To address this issue, we develop a memory efficient scheme by exploiting the highly confined foci in the PSF image. Specifically, we remove most of the dark regions in the PSF images and then align the cropped foci. The alignment step essentially compensates for the depth-dependent lateral shift in the off-axis foci. We denote this “compressed” and aligned PSF calibration set as \( H_c(u', v'; x, y, z) \), where \( (u', v') \) are the new pixel coordinates after cropping and alignment.

2) We approximate the \( N \) calibrated PSFs by a rank-K decomposition via a truncated singular value decomposition (SVD):

\[
H_c(u', v'; x, y, z) \approx \sum_{i=1}^{K} M_i(x, y, z) H_{b_i}(u', v'),
\]

where \( H_{b_i}(u', v'), \{i = 1, ..., K\} \) denotes the \( i^{th} \) basis PSF and \( M_i(x, y, z), \{i = 1, ..., K\} \) is the corresponding coefficient volume. Equation (1) essentially approximates the set of...
calibrated PSFs as a linear combination of $K$ basis PSFs. The first five basis PSFs and the matching coefficient volumes are visualized in the first two rows in Fig. 3c. We choose $K = 64$, which has a small $\sim 2.5\%$ approximation error on the calibration set, as shown in Fig. 3d. In practice, the choice of $K$ incurs a tradeoff between the model accuracy and computational cost. In addition, this low-rank approximation also helps suppress noise in the raw PSF measurements.

![Sparse PSF calibration](image)

![Calibrated 3-LSV PSF data set](image)

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| Basis PSFs | | | | | |
| Calibrated coefficient volumes | | | | | |
| 3D-interpolated coefficient volumes | | | | | |

![Approximation error](image)

![Validation of 3D-LSV model at an unseen location](image)

Figure 3: **3D Linear Shift Variant (LSV) model of the CM$^2$.** (a) Illustration of the sparse PSF calibration process. A 5-μm point source is scanned through the $8 \times 8 \times 1$ mm$^3$ imaging volume with a lateral step size of 1 mm and axial step size of 100 μm, generating in-total 891 calibrated PSFs. (b) Example preprocessed calibrated PSFs. The shift variance in 3D is clearly visible. (c) Results of the low-rank decomposition. Row 1-2: the computed basis PSFs and the coefficient volumes respectively from the decomposition on the calibrated PSFs. Row 3: the 3D-interpolated coefficient volumes. (d) $K = 64$ basis PSFs are empirically chosen for our 3D-LSV model, which yields a small 0.025 normalized mean squared error (MSE). (e) Validation of the simulated PSF using our 3D-LSV model at an unseen location. The error between the numerically calculated and experimentally measured PSFs is small, as quantified by the pixel-wise absolute error.

(3) To obtain the coefficient volume for the corresponding basis PSFs at any uncalibrated 3D locations, we further perform 3D bilinear interpolation for all the coefficient volumes from the sparse calibration grid onto the dense reconstruction grid. The interpolated coefficients at any arbitrary 3D locations $(x_i, y_j, z_k)$ is denoted as
The validity of this procedure relies on the assumption that the PSFs are slowly varying in 3D. This means that 1) the basis PSFs can be accurately estimated from a sparse set of PSF measurements, and 2) the decomposition coefficients are smooth in 3D. A detailed theoretical treatment is described by Debarnot et al. The interpolated coefficient volumes for the first five basis PSFs are shown in the third row in Fig. 3c.

(4) To generate a synthetic CM² measurement, we compute k weighted 2D depth-wise convolutions in the lateral dimension \( \odot_{u,v} \), followed by a summation along the axial dimension \( z \):

\[
g(u,v) \approx \sum_z \sum_{i=1}^{k} [M^i(u,v,z)O(u,v,z)] \odot_{u,v} H^i_b(u,v,z).
\]

(2) Here, \( O(u,v,z) \) is the 3D fluorescence intensity distribution of the imaging volume. \( M^i(u,v,z) \) is the \( i \)-th coefficient volume. \( H^i_b(u,v,z) \) is the corresponding basis PSF, which has been placed back to the original sensor pixel coordinates by accounting for the expected lateral shift at each depth \( z \). The pixel coordinates \( (u,v) \) in the image space and the object space coordinates \( (x,y) \) are simply related by the magnification \( M \) of the CM²: \( u = Mx \), \( v = My \).

![Figure 4: Experimental validation of the 3D-LSV model. (a) Simulated measurements using our 3D-LSV model and the depth-wise LSI model (sample: 10-μm fluorescence beads randomly placed in a 5 x 5 x 0.8 mm³ volume). (b) Experimental measurement on 10-μm fluorescence beads with similar density to the simulation. This comparison shows that our 3D-LSV model can accurately capture the key aberrated features in real experiments.](image)

To experimentally validate the accuracy of this 3D-LSV, we compare the numerically simulated and physically measured PSFs at an “unseen” off-axis location in Fig. 3e. Notably, the simulated PSF accurately captures the off-axis aberrations resulting in the unevenly distributed focus intensities and the foci’s irregular shapes. Moreover, to visually validate the synthetic measurement generated by this 3D-LSV model, we compare a simulated image and an experimental measurement taken from a 5 x 5 x 0.8...
mm³ volume with 10-µm fluorescence beads with similar seeding density in Fig. 4. The forward model used in our V1 system is based on a depth-wise linear shift invariant (LSI) approximation. To highlight the improvement of the proposed 3D-LSV model over the depth-wise LSI model, we compare the synthetic measurements from the same object using both models in Fig. 4a. In the zoom-in panels taken from the same regions at the peripherical FOV where the aberrations are more apparent, we highlight that the 3D-LSV model can synthesize the key image features in the real experiment. This is essentially important to train CM²Net based on only simulation data, yet the trained network is directly generalizable to real experimental measurements.

2.3 CM²Net

To enable fast and accurate 3D reconstruction from a single-shot CM² measurement, we develop a deep learning model. This deep neural network, termed CM²Net, incorporates several key characteristics of the CM² physical model into its architecture design. In this section, we highlight a few insights in the network design and refer the implementation details to Section 4.5 and Supplementary Information Section S4.

Figure 5: CM²Net network structure. The raw CM² measurement is first preprocessed to form a multiplexed view stack. The demixing-net removes the crosstalk artifact and outputs the demixed view stack by learning characteristic view-dependent aberrations. The demixed view stack is processed by the “shift-and-add” lightfield refocusing algorithm to form a geometrically refocused volume. The enhancement-net branch removes the refocusing artifacts and enhances the reconstructed 3D resolution. The view-synthesis-net branch directly processes the demixed views to perform the 3D reconstruction. The final output is the sum from the two branches. The CM²Net is trained with a mixed loss function combining the demixing loss and the reconstruction loss.

A key feature of the CM² image formation is the view-multiplexing that contains 3 × 3 tomographic projection information about the 3D object. This multi-view imaging geometry introduces two main challenges to the network design. First, the image features needed for performing 3D reconstruction are non-local, instead they are separated by a few thousands of pixels in each image. However, to fully capture the non-local information requires a sufficiently large receptive field, which is not easily achieved by a standard convolutional neural network (CNN) building on small-sized convolution kernels. Second, the view-multiplexing requires the network not only to reconstruct 3D information based on the depth cues, but also to remove crosstalk artifacts from the view multiplexing based on other image features. To address these challenges, CM²Net combines three network modules to break the highly ill-posed inverse problem into three
simpler tasks, including view-demixing, view-synthesis, and lightfield-refocusing enhancement, as illustrated in Fig. 5. Working in synergy, CM²Net provides two major improvements over the model-based reconstruction algorithm used in our V1 system\textsuperscript{28}, including achieving an axial resolution of \(~25\mu m\) (~8\times better) and reconstructing an \(8 \times 8 \times 1\) mm\(^3\) volume in <4 seconds (~1400\times faster).

The first module is the view "demixing-net". It demultiplexes each CM\(^2\) image into nine individual "demixed" views, each corresponding to the image captured by a single microlens alone without the crosstalk from other microlenses in the array. To perform this task, the demixing-net must synthesize the information contained in all the views across the entire CM\(^2\) measurement. To facilitate this process, we first construct a "view stack" consisting of nine cropped and "view-aligned" image patches from the raw CM\(^2\) measurement as the input to the demixing-net, as illustrated in Fig. 5. The view alignment is performed by aligning the chief ray of each microlens at the image plane (see details in Section 4.4). This input view stack still contains multiplexed information, which the demixing-net seeks to demultiplex. The ground-truth output for the training is the demixed view stack containing nine crosstalk-free images. The training of the demixing-net is made only possible on \textit{simulated} training data using our 3D-LSV model since it is impractical to experimentally collect a large set of paired multiplexed and demixed image-stacks. This highlights another advantage of our simulator-based training strategy. Our results show that this task can be accurately performed by the demixing-net by utilizing the distinctive aberration features from different microlenses. In Section 2.4, we further highlight that the demixing-net helps significantly reduce the reconstruction artifacts and enables more robust 3D reconstructions.

The demixed view stack is akin to a \(3 \times 3\) view lightfield measurement. As such, we design the reconstruction module consisting of two branches to process the demixed views in parallel. The first branch is a "view-synthesis-net", which directly performs the 3D reconstruction based on view disparity information in the input. The effectiveness of this pure data-driven approach has been demonstrated in several LFM systems\textsuperscript{35–37}. The second branch explicitly incorporates the geometrical optics model in lightfield. The demixed views are first process by the lightfield refocusing algorithm\textsuperscript{45} to generate a refocused volume. The refocused volume is then fed into the lightfield "enhancement-net" to remove artifacts and enhance the reconstructed resolution. The outputs from the two branches are summed to yield the final 3D reconstruction. The intuition of this design is that view-synthesis-net learns to compensate for the residual error from the enhancement-net's output, similar to residual learning\textsuperscript{46}.

Overall, the CM\(^2\)Net contains three learnable sub-networks, including demixing-net, view-synthesis-net and enhancement-net, and a fixed non-learnable lightfield refocusing layer. The CM\(^2\)Net is trained entirely on the simulated data. The loss function combines the demixing loss and the reconstruction loss, which promotes the fidelity of the demixed views and the 3D reconstruction results, respectively. Additional information on the synthetic training data and the implementation details of CM\(^2\)Net are provided in Sections 4.4, 4.5 and S4.

2.4 View-demixing network module suppresses reconstruction artifacts

In this section, we show in simulation that the view-demixing task can be reliably performed and the demixing-net module substantially improves the quality in the downstream 3D reconstruction.
Figure 6: **View-demixing network module significantly improves 3D reconstruction quality.**

(a) View demixing results on a simulated CM² measurement (sample: fluorescent beads with random sizes between 10 µm and 20 µm in a cylindrical volume (7-mm diameter, 0.8 mm depth). The multiplexing artifacts in the “input view” from the raw CM² measurements are effectively demultiplexed by the demixing-net. (b) Ablation study results on the demixing-net. The XY MIPs shows that the proposed CM²-Net structure with the demixing-net module provides high-quality reconstruction. Removing the demixing-net module results in a large number of false positives (marked by yellow arrows) in the reconstruction.

First, we demonstrate the effectiveness of view-demixing by comparing the demixing-net demixed views against the ground truth in simulation. Both the CM² measurement and the nine individual crosstalk-free views are simulated using our 3D-LSV model using the PSFs of the MLA and each microlenses, respectively (see details in **Section 4.4**). In **Fig. 6a**, we show results on a testing volume consisting of fluorescent beads with random sizes between 10 µm and 20 µm in a cylindrical volume (diameter ~7 mm, thickness ~0.8 mm). The large FOV results in strong view multiplexing in the raw CM² measurement, as evident in the example “input view” centered around the central microlens. The “demixed
view” from the demixing-net closely matches with the ground-truth view from the central microlens without crosstalk artifacts, as highlighted in the three zoom-in panels taken from widely separated regions. The demixing-net can robustly perform this task since each fluorescent bead imaged by different microlenses contain distinct aberrated features.

Next, we show that the view-demixing step significantly improves the 3D reconstruction quality. Specifically, we perform the following ablation study in Fig. 6b. The same dual-branch reconstruction network (including the lightfield-refocusing enhancement module and the view-synthesis module) is used to process either the demixing-net demixed views or the multiplexed views from the raw measurement on the same simulated data. The XY maximum intensity projections (MIPs) of the 3D reconstructions from the two networks and the ground-truth volume are shown in Fig. 6b. As highlighted in the two zoom-in regions, the reconstruction from the network with the demixing-net closely matches with the ground truth, whereas the one without view-demixing suffers from a large number of false positives (marked by the yellow arrows).

We further quantify the reconstruction quality by recall and precision (details about the evaluation metrics in Section 4.8). The CM²Net with the demixing-net achieves an average recall and precision of 0.7 and 0.94 respectively, while the one without the demixing-net achieves much lower recall of 0.57 and precision of 0.24. This comparison highlights that although the CM²Net without the demixing-net is sensitive enough to recover 57% of the emitters, the 3D reconstruction suffers from very low precision. The comparison on the precision values implies that the demixing-net helps to reduce the false-positive rate from 0.76 to 0.06, a ~13× improvement. We conduct a more thorough evaluation of the CM²Net performance in Section 2.6.

2.5 3D-LSV simulator enables accurate 3D reconstruction across a wide FOV

In this section, we show that the 3D-LSV simulator developed in Section 2.2 is essential to achieve fast and accurate 3D reconstruction across a wide FOV by CM²Net.

Our CM²Net is trained entirely on simulated data. To demonstrate the importance of incorporating the 3D-LSV model in the training data, we compare two networks using the same CM²Net architecture (Fig. 5), but are trained with two different data sets. The first set is simulated by our 3D-LSV model. The second set is simulated by the depth-wise LSI model used in V1 system 28, which assumes that the PSF measured from an on-axis point source is invariant at each depth. To make a fair comparison, the two data sets are generated from the same set of synthetic volumes (more details in Section 4.4). In the following of this section, we denote the CM²Net trained with the 3D-LSV data set as LSV-CM²Net, while the network trained with the depth-wise LSI data set as LSI-CM²Net.

The 3D reconstructions on a cylindrical volume (7-mm diameter, 0.8-mm depth) from LSV-CM²Net and LSI-CM²Net are shown in Fig. 7a and 7b, respectively. In addition, in Fig. 7c we also show the result from the depth-wise LSI model-based 3D deconvolution algorithm used in our V1 system 28. In each figure, we overlay the reconstruction (in red) onto the ground truth (in green) and visualize the XY and XZ MIPs. When the reconstruction matches with the ground truth (i.e. True Positives), the overlaid region appears in yellow. When the reconstruction misses certain particles (i.e. False Negatives), the region appears in green. When the reconstruction creates false particles (i.e. False Positives) or suffers from axial elongations due to the missing cone artifacts, the region appears in red.
Figure 7: The 3D-LSV simulator enables wide-FOV, high-resolution 3D reconstruction. 3D reconstructions from (a) LSV-CM²Net trained with the 3D-LSV simulator, (b) LSI-CM²Net trained with the depth-wise LSI simulator, and (c) depth-wise LSI model-based 3D deconvolution. LSV-CM²Net provides accurate and high 3D resolution reconstruction across the entire volume. In comparison, the LSI-CM²Net suffers from many false negatives while the model-based deconvolution suffers from severe axial elongations. In all figures, the positive z-axis points at the direction towards the CM² system.

By visually inspecting the MIPs of the reconstruction, we conclude that the LSV-CM²Net can accurately reconstruct the entire 7-mm FOV throughout the 0.8-mm depth range. This is highlighted by the three zoom-in regions of the XZ MIPs taken from the central and two peripheral regions in Fig. 7a. In contrast, the LSI-CM²Net reconstruction suffers from severe artifacts especially beyond the central 3-mm diameter region, as shown in Fig. 7b. This result shows that the depth-wise LSI simulator severely limits the network’s ability to generalize to realistic CM² measurements. The network is particularly sensitive to the distributional mismatch between the LSV and LSI data sets.

We also benchmark the network reconstructions against the previously established model-based deconvolution method. Similar to our previous report, the model-based reconstruction matches well with the ground truth across the entire volume. However, it suffers from severe axial elongations (i.e. missing cone artifacts) due to the small numerical aperture (NA) of the CM² platform. This is clearly visible in the reconstruction’s XZ MIPs in Fig. 7c.

A major improvement of the LSV-CM²Net over the model-based reconstruction is its ability to significantly reduce the axial elongations, as shown in the XZ MIPs in Fig. 7a. Additional quantitative comparisons in real experiments are presented in Section 2.7. An additional practical benefit of the deep learning algorithm is its much-reduced reconstruction time and memory burden. To perform the large-scale reconstruction in Fig. 7 (~230 million voxels required to discretize the imaging volume), the model-based reconstruction using the iterative ADMM algorithm requires ~1.4 hours and ~150-GB RAM for each volume. In contrast, the CM²Net takes only ~3.6 seconds on an entry-level GPU (Nvidia RTX 2070, 8GB RAM), which is ~1400× speed-up and ~19× memory cost reduction.
2.6 Quantitative analysis of CM²Net performance

In this section, we quantitatively evaluate the performance of CM²Net under different imaging conditions on simulated data sets generated using our 3D-LSV simulator. Our results show that the trained CM²Net can provide high-quality reconstruction and is robust to variations in the FOV size and fluorescent emitter’s size, intensity, 3D location and seeding density.

To perform the evaluation, we simulate a testing set consisting of nine density ranges in [10:10:100] (number of emitters/mm²), which matches the neuronal density that can be recorded by the state-of-the-art one-photon table-top mesoscope⁶. Within each density range, we synthesize 20 volumes with randomly distributed emitters. In addition, to show how CM²Net performs under severe view-multiplexing at around 7-mm FOV, the synthetic volumes are generated with random FOVs whose values are uniformly distributed between 6.5 mm and 7.5 mm. The degree of view-multiplexing in CM² is directly determined by the FOV². For FOV < 2.7 mm, no view-multiplexing is present and the CM² measurement consists of 3 × 3 non-overlapping views of the object. As the FOV increases, the amount of overlap between the neighboring views increases accordingly. At the largest FOV = 7.5 mm, the amount of overlap between neighboring views is approximately 64%. Each volume axially spans the [-400 µm, 400 µm] range, with the nominal focal plane at z = 0 µm, and a positive depth is closer to the MLA. We simulate fluorescent emitters with random diameters uniformly distributed between 8 µm and 20 µm, which covers the common size range of neuronal cell bodies. The emitter’s intensity is set by the surface area, i.e. proportional to the diameter squared. In addition, a random scalar is multiplied to each emitter’s intensity, which follows a uniform distribution between 0.8 and 1.2. This ± 20% variation range is chosen based on the typical contrast captured in one-photon Calcium imaging during neuronal activations⁴⁷. Additional details about synthetic data generation are provided in Section 4.4.

We quantify the detection capability of CM²Net using recall (a.k.a. sensitivity) and precision (details in Section 4.8). Recall measures the sensitivity / detection rate of CM²Net by the ratio between the correctly reconstructed and the actual total number of emitters. Precision measures the specificity by the ratio between the correctly reconstructed and the total reconstructed number of emitters. We choose this set of metrics because they focus on quantifying the error made on the reconstructed emitters, whereas voxel-value averaged metrics, e.g. mean squared error, are biased by the background for sparse objects like ours. We measure the detection performance when varying all four possible parameters of interest, including the emitter’s lateral location, density, depth, and size. When calculating the statistics at a given condition (e.g. a lateral location), all other parameters (e.g. emitter’s depth, density, size) are randomized.

First, the performance for emitters at different lateral locations are evaluated in Fig. 8a. In each plot, we aggregate all the emitters into 7 sample sets according to their lateral distances from the center of the FOV including [0 mm: 0.5 mm: 3.5 mm], and then compute the mean and standard deviation of each metric for each set. The averaged precision (in blue) and recall (in orange) remain higher than 0.93 and 0.68 when the lateral distance is < 3 mm (i.e. FOV < 6 mm). The precision and recall reduce to ~0.85 and ~0.37 when the lateral distance is ~3.5 mm (FOV = 7 mm). The standard deviation (shown as the error bar) increases as the lateral distance, indicating that the reconstruction is more consistent at the central FOV region. To provide a better visualization, we calculate the recall and precision maps in Fig. 8a (see details in Section 4.8). The precision map shows that CM²Net provides nearly isotropic, high specificity within the 7-mm FOV. Meanwhile, the recall map shows that CM²Net provides a high detection rate in the central 6-mm FOV, and then degrades at the outer FOV.
Figure 8: Quantitative evaluation of CM2Net at different conditions in simulation. Detection rate measured by recall when varying emitter's (a) lateral location, (b) seeding density, (c) depth, and (d) size. (a) CM2Net achieves consistently high precision above ~0.85 for lateral location < 3.5 mm (FOVs < 7 mm). The recall drops from 0.97 at the central to 0.35 near the edge of FOV. The recall and precision maps (250 μm patch size) show nearly isotropic, high detection performance across the central 6-mm FOV. (b) The precision is consistently > 0.92 for all emitter densities. The recall decreases from ~0.83 at the lowest density (10 emitters/mm²) to ~0.61 at the highest density case (100 emitters/mm²). Example CM² image patches at 15, 55, 95 emitters/mm² are shown in the top panel to visualize the density variations. (c) The precision is consistently > 0.95, and the recall is > 0.7 within the [-400 μm, 200 μm] range. The slight decrease of recall in the [200 μm - 400 μm] range (closer to the MLA) is due to the more aberrated PSFs. The foci from the central microlens at -400 μm, 0 μm, and 400 μm are shown in the top panel to visualize the depth-dependent aberrations. (d) The precision is > 0.9, and the recall is > 0.71 for emitter's diameter > 11 μm. Both the precision and recall degrade for smaller emitters since the local SNR and the emitter's intensity approximately scales with the size squared. The top panel shows the XY MIPs of reconstructed emitters of size [8, 14, 20] μm.

To better understand the origin of this performance degradation, we perform the following ablation study in Section S5. First, we train another network with only the reconstruction module of the CM²Net that takes the ground-truth demixed views as the input. The reconstruction results achieve recalls close to 1 consistently for the entire 7-mm FOV range (see Fig. S6a). Next, we directly feed the ground-truth demixed views to the reconstruction module of the trained CM²Net. The results show slightly decreased recall as compared to that from the retrained reconstruction module but remains >0.89 across the 7-mm FOV (see Fig. S6a). Together, this study shows the effectiveness and robustness of CM²Net’s reconstruction module. The degraded performance at the outer FOV is due to imperfect view-demixing at these regions. To further diagnose the system, we compare the intensity distribution map of the point source used for our PSF calibration and the recall map in Fig. S6b, and find qualitative correspondence. We
hypothesize that because of the large PSF intensity drop at the peripheral FOV (~85% drop at the edge of the 7-mm FOV), the view-demixing task is harder to achieve for these dimmer sources.

The metrics for different emitter densities are evaluated in Fig. 8b. We aggregate the statistics in 9 testing sets with [10: 10: 100] (emitters / mm$^2$) density ranges. Example measurements corresponding to 15, 55, 95 (emitters / mm$^2$) are shown in the top panel. As expected, both the precision and recall generally decrease as the density increases. The precision remains >0.92 for all emitter densities, indicating very few false positives in the reconstruction despite the large (1 order of magnitude span) density variations tested. The recall decreases approximately linearly from ~0.83 at the lowest density (10 emitters/mm$^2$) to ~0.61 at the highest density case (100 emitters/mm$^2$). This means that as the density increases, CM$^2$Net suffers from more mis-detections (i.e. false negatives).

The metrics for different emitter’s depths are quantified in Fig. 8c. We compute the statistics at 8 depth ranges including [-400 µm: 100 µm: 400 µm]. The precision is consistently >0.9 for the entire [-400 µm, 400 µm] range. The recall is >0.7 within the [-400 µm, 200 µm] range, and gradually decreases to ~0.6 at the 400 µm depth. To explain this observation, we also visualize the on-axis PSFs of the central microlens at z = -400 µm, 0 µm, and 400 µm respectively in the top panel. As the source moves closer to the MLA, the PSF is degraded more severely by the defocus aberrations. This results in lower SNRs in the raw measurement, which in turn leads to increased mis-detections.

Finally, we quantify the metrics for different emitter diameter ranges including [7 µm: 2 µm: 21 µm]. For the emitters ranging 11-20 µm, the precision is >0.9 and the recall is >0.71. As the emitter size decreases, both the precision and recall drop approximately linearly to 0.55 and 0.48 for the smallest 8-µm emitters, respectively. We attribute the degraded performance for smaller emitters to two main factors. First, since the emitter’s intensity is roughly proportional to the size squared, the SNR rapidly decreases as the size reduces. Second, due to the poor sampling (the lateral and axial voxel size is 4.15 µm and 10 µm respectively), the number of voxels representing each emitter is <5 when the emitter size is <11 µm (as shown in the top panel of Fig. 8d).

The averaged precision and recall calculated on the entire testing set is ~0.7, ~0.94 respectively, which comparable to the state-of-the-art deep learning-based neuron detection pipeline$^{48}$. This study establishes that CM$^2$Net can detect the fluorescent emitters with very few “hallucinated” sources (~4% false positive rate on average) and relatively high detection rates (~30% mis-detection rate on average) for a broad range of conditions.

### 2.7 CM$^2$Net achieves high 3D resolution, wide-FOV reconstruction in experiments

A major achievement we demonstrate here is that the generalization capability of the simulator-trained CM$^2$Net enables high 3D resolution reconstruction in real experiments with high detection performance.

We first image a cylindrical volume (diameter ~6.7 mm, thickness ~0.5 mm) embedded with 10-µm green-fluorescent beads (more details in Section 4.6). The phantom we used in this experiment is estimated to have 10-20 emitters / mm$^2$. The raw CM$^2$ measurement is first pre-processed (more details in Section 4.7) and then fed into the CM$^2$Net to perform 3D reconstruction. The CM$^2$Net reconstruction is shown in Fig. 9a with the raw CM$^2$ measurement shown in the insert. The 3D reconstruction is validated against the widefield measurements taken by a standard table-top epi-fluorescence microscope in Fig. 9b. First, we validate the full-FOV reconstruction by
comparing the XY MIPs of the CM²Net 3D reconstruction and the widefield z-stack measurement with a 2×, 0.1 NA objective lens (more details in Section 4.6). To further assess the reconstruction at greater resolution, two zoom-in XY MIPs of the network reconstruction taken from the central and the edge of the FOV are compared with high-resolution z-stack measurements with a 20×, 0.4 NA objective lens. By visual inspection, the network reconstruction matches well to the widefield measurements. The reconstruction quality does not degrade significantly at peripheral FOV regions, a marked improvement over our depth-wise LSI model-based reconstruction in V1 system28, benefited from our 3D-LSV simulator-based training.

A major goal we aim to achieve using the CM² is high-resolution imaging across a wide FOV. To highlight this capability, we compare the FOVs achieved by the CM² V2 (~7 mm) and the FOVs of the 2× objective (~8 mm) and 20× objective (~800 µm) on our table-top microscope, as also indicated in Figs. 9a and 9b. Representative axial profiles of the 10-µm beads reconstructed by CM²Net, the model-based deconvolution, and 2× and 20× widefield measurements are compared in Fig. 9d. The axial elongation of each method is measured by the FWHM of a Gaussian fit to the axial profile. Notably, CM²Net achieves an axial elongation of ~24 µm, which is ~8× better than the model-based deconvolution28 (~184 µm) and outperforms 20×, 0.4 NA measurement (~39.7 µm).

To quantitatively evaluate the detection performance, we compute recall, precision, and F1-score by comparing the XY MIPs of the CM²Net reconstruction and the widefield 2× measurement (details in Section 4.8). The CM²Net reconstruction achieves a recall ~0.78 and precision ~0.80. In comparison, the recall and precision in simulation at the corresponding density range is ~0.83 and ~0.97 respectively (Fig. 8b). This shows that the simulator-trained CM²Net degrades slightly on experiments with ~5% higher false positive rate and ~17% higher mis-detection rate at this imaging condition. We attribute the reduced performance to the undesired extra views in the experimental measurements due to an extra column of partial microlenses adjacent to the main 3 × 3 microlens array (see Fig. S8).

To further provide more spatially fine-grained quantitative assessment, we construct the recall and precision maps in Fig. S9a by calculating the patch-wise (500 × 500 µm² patch size) local recall and precision values. The recall in most of the regions are >0.75, which indicates a <25% mis-detection rate. The precision is consistently >0.8 except for a few patches that contain fewer than 2 beads, which indicates only a few false positives in the reconstruction. In Fig. 9c, we show the F1-score map as a combined measure of local precision and recall to summarize the CM²Net detection performance. The F1-score map generally achieves a high value of >0.75. We also label the region with F1 = 0, which indicates either the widefield measurement or the CM²Net reconstruction contains no beads at the patch (more details in Section S7). An overlay between the full-FOV CM²Net reconstruction and the widefield 2× measurement is shown in Fig. S10b to provide further visual inspections.

This experiment shows that the CM²Net trained with our 3D-LSV simulator can provide high 3D resolution reconstruction across a wide FOV with high sensitivity and precision. Notably, the 24-µm axial elongation achieved by CM²Net is ~4× better than the 100-µm axial spacing used in the PSF calibration. This shows that the axial interpolation implemented in our 3D-LSV model is highly effective to achieve axial “super resolution” even in real experiments. Both the sensitivity and precision agrees with the simulation, validating the CM²Net that trained purely on synthetic data can generalize well to experimental measurement.
Figure 9: **CM²Net's achieves high 3D resolution reconstruction across a wide FOV in experiments.** (a) Visualization of the CM²Net reconstruction. The raw CM² measurement shown as the insert. (b) Validation experiments taken from 2×, 0.1 NA and 20×, 0.4 NA objective lenses on a table-top widefield (WF) microscope. CM²Net provide high-quality reconstruction across the entire 7-mm FOV as benchmarked by the 2× measurement. Both lateral and axial reconstructions are in good agreement with the high-resolution 20× measurement (sample: 10-µm fluorescent beads in a cylindrical volume with ~6.7-mm diameter and ~0.5-mm depth). (c) F1-score map computed by comparing the XY MIPs of the CM²Net reconstruction and widefield 2× measurement on non-overlapping 500 × 500-µm² patches. The x labeled patches mean the F1 score is zero, which is because either the widefield measurement or the CM²Net reconstruction is empty. (d) The axial profiles from the CM²Net reconstruction compared with the WF measurements using the 2× and 20× objective lenses and the model-based deconvolution. The axial elongations are 195 µm, 184 µm, 39.7 µm, and 24.4 µm for WF 2×, model-based deconvolution, WF 20×, and CM²Net reconstruction, respectively. This shows that the CM²Net provides superior axial localization.
2.8 Experimental demonstration on mixed size fluorescent beads

In real experiments, fluorescent emitters with different sizes and brightness result in different local contrast and SNRs in the raw CM² measurement\(^2\). This is an important consideration as we develop CM² towards realistic biological applications. To demonstrate this capability, we conduct proof-of-concept experiments on mixed size fluorescent beads. Our result shows that CM²Net can robustly handle such sample variations in real experiments.

We image a cylindrical volume (diameter ~6.5 mm, depth ~0.8 mm) embedded with mixed 10-µm and 15-µm green-fluorescent beads (details in Section 4.6). The phantom we used in this experiment is estimated to have 10-20 emitters / mm\(^2\). In the CM² raw measurements, the 15-µm beads are ~2.2× brighter than 10-µm beads, matching with the ratio of their surface areas and the model used in our synthetic data. The CM²Net reconstruction is shown in Fig. 10a with the raw CM² measurement shown in the insert. The 3D reconstruction is validated against the widefield measurements on a table-top epi-fluorescence microscope in Fig. 10b. First, we assess the full-FOV reconstruction by comparing the XY MIPs of the CM²Net 3D reconstruction and the widefield z-stack measurement with a 2×, 0.1 NA objective lens. We further compare the reconstruction details on two zoom-in regions from the center and corner FOVs with the high-resolution z-stack taken by a 20×, 0.4 NA objective lens. By visual inspection, CM²Net reliably reconstructs both 10-µm and 15-µm beads and the result matches well with the widefield measurements. Like our previous result on mono-size (10-µm) beads, CM²Net achieves superior axial localization. The XZ MIPs of the CM²Net 3D reconstruction are in good agreement with the z-stack measurements from the 20×, 0.4 NA objective lens in the zoom-in regions. The axial confinement on both 10-µm and 15-µm beads by CM²Net are better than the widefield 20×, 0.4 NA measurements.

To quantitatively evaluate the quality of the CM²Net reconstruction, we compute the recall, precision, and F1-score maps in Fig. S10a by comparing the XY MIPs from the CM²Net reconstruction and widefield 2× measurement (details in Section 4.8). The CM²Net achieves averaged recall ~0.73 and precision ~0.84 on the mixed size beads across the entire 6.5-mm FOV. As compared to the mono-10-µm bead experiment, we hypothesize that the decreased recall is attributed to the greater intensity and SNR variations caused by bead size variations in the measurement. The increased precision is attributed to the reduced FOV and less contaminations from the extra views (as shown Fig. S10). An overlay between the full-FOV CM²Net reconstruction and the widefield 2× measurement is shown in Fig. S10b to provide further visual inspections. The results show that CM²Net is robust to the emitter size and intensity variations in experiments.

This experiment again highlights the wide-FOV and high-resolution 3D imaging capability achieved by CM² V2. Our training data containing randomized emitter sizes and intensities are effective to make CM²Net robust to the experimental variations. As a result, CM²Net can provide high-quality 3D reconstruction with good sensitivity and precision on mixed size fluorescent emitters that have large differences in the feature size and local SNR.
Figure 10: Experimental result on mixed fluorescent beads. (a) Visualization of the CM²Net reconstruction. The raw CM² measurement shown as the insert. (b) Validation experiments taken from 2×, 0.1 NA and 20×, 0.4 NA objective lenses on a table-top widefield microscope (sample: mixed 10-µm and 15-µm fluorescent beads in a cylindrical volume with ~6.5-mm diameter and ~0.8-mm depth). The CM²Net full-FOV reconstruction is in excellent agreement with the 2× measurement. The lateral and axial reconstructions are validated by the high-resolution 20× measurement in both central and peripheral FOV regions.
3 Conclusion and Discussion

In summary, we have presented a new Computational Miniature Mesoscope (CM$^2$) V2 system, which is a deep learning-augmented miniaturized microscope for single-shot 3D high-resolution fluorescence imaging. The new system reconstructs fluorescent emitters across a $\sim$7-mm FOV and 800-$\mu$m depth with high sensitivity and precision, and achieves $\sim$7-$\mu$m lateral and $\sim$25-$\mu$m axial resolution.

The main hardware advancement in CM$^2$ V2 includes a novel 3D-printed freeform illuminator that increases the excitation efficiency by $\sim$3x. Each 3D-printed LED collimator can provide up-to 80% light delivery efficiency yet weighs only 0.03 grams. It is low-cost and rapidly fabricated on a table-top 3D printer. In addition, we adapted a hybrid emission filter design that suppresses the excitation leakage and improves the measurement SBR by more than 5x.

The main computational advancement includes three main parts. First, we developed an accurate and computationally efficient 3D-LSV forward model that characterizes the spatially varying PSFs across the large ($cm^2 \times mm$-scale) imaging volume supported by the CM$^2$. Second, we developed a multi-module CM$^2$Net that achieves robust, high-resolution 3D reconstruction from single-shot CM$^2$ measurement. Third, using the 3D-LSV simulator to generate the entire training data set, the trained CM$^2$Net provides high detection sensitivity and precision and superior axial localization on fluorescent emitters across a wide FOV and at a variety of conditions, and more importantly generalize well to real experiments.

Our pilot demonstration on the utility of freeform optics fabricated by 3D printing may be a fruitful area for future research, especially for miniature microscopes and other miniature optical devices. In recent years, freeform optics has emerged as the ideal solution to bypass many limitations in conventional optics, such as compactness and imaging performance$^{29}$. At the same time, non-conventional optics has been enabled by novel 3D printing process, such as GRIN lens$^{49}$, micro-optics$^{22,50}$, diffractive optics$^{51}$, and volume optics$^{52}$. Based on these exciting developments, it can be envisioned that additional 3D-printed freeform optical components can be incorporated in future generation of the CM$^2$ platform to further enhance its imaging capability.

The CM$^2$ V2 platform is built on a back-side illuminated (BSI) CMOS sensor, which significantly improves the measurement’s SNR and dynamic range over the conventional CMOS sensor in the V1 platform. The size and weight of the CM$^2$ V2 prototype is limited by the availability of miniature BSI CMOS sensor. However, we do not anticipate this to be a major roadblock for future development as encouraged by the recent development of MiniFAST$^{53}$ BSI CMOS-based miniscope. With further advancement on high-speed data transmission and high pixel-count BSI CMOS sensor platform, we envision that future generations of CM$^2$ can be further miniaturized to be suitable for “wearable” $in$-vivo neural recordings on mice and other small animals.

Our 3D-LSV model is essential to achieve high 3D resolution reconstruction across a large imaging volume. A notable result we have demonstrated is that the axial resolution is not limited by the axial step used for the 3D PSF calibration. This allowed us to bypass the large data requirement in the alternative depth-wise LSV framework$^{22,25,34}$ and to perform data-efficient PSF calibrations across a centimeter-scale FOV and millimeter-scale depth range. We expect the same sparse 3D PSF calibration, low-rank decomposition, and 3D interpolation procedure are applicable to other computational 3D microscopy techniques, such as LFM$^{15,17,45}$ and lensless imaging$^{23,24,54}$.

Our CM$^2$Net architecture is designed to incorporate both the view-multiplexing and lightfield information in the CM$^2$ image formation. It combines view-demixing, lightfield-
refocusing enhancement, and view-synthesis modules. We have shown that the view-demixing module significantly suppresses the false positives in the 3D reconstruction. The simulator-training scheme was essential to enable the training of the view-demixing sub-network. This highlights several key advantages of simulator-based training over experiment-based training schemes. It not only forgoes the laborious physical data collection process, but also enables access to novel data pairs that are impractical to collect experimentally. The reconstruction module combining the lightfield-refocusing enhancement and view-synthesis modules is shown to provide highly accurate reconstructions, and is readily applicable to other LFM modalities to enhance the 3D reconstruction quality.

An outstanding challenge to expand the utility of the CM\(^2\) is tissue scattering. There are several promising solutions we envision that are applicable to the CM\(^2\) system, such as miniature structured illumination microscopy technique and scattering-incorporated 3D reconstruction frameworks, which will be investigated in our future work.
4 Materials and Methods

4.1 The CM² V2 design and prototype

The CM² V2 platform consists of two main parts, including the imaging module and the illumination module. The overall design is visualized in Figs 1a and 2a. Built from 3D-printed components and off-the-shelf optics and electronics, the CM² V2 platform has an overall dimension of 36 mm × 36 mm × 15 mm including the backside-illuminated (BSI) CMOS PCB board (IDS Imaging, monochrome BSI CMOS IMX178LLJ, 2076 × 3088 pixels, 2.4-µm pixel size, 12-bit dynamic range, 58 fps). The entire assembled CM² V2 prototype weighs ~11.5 grams, of which the CMOS PCB takes most of the size (36 mm × 36 mm × 4 mm) and the weight (9 grams) whereas the custom parts weigh only 2.5 grams in-total.

In the imaging module, we use an off-the-shelf rectangular MLA with a 100% fill factor (Fresnel Technologies Inc., no. 630, focal length = 3.3 mm, lens pitch = 1 mm, thickness = 3.3 mm). The MLA is directly placed on top of the CMOS sensor to achieve single-shot 3D imaging with a compact form factor. The MLA is diced into a 3 × 3 array using a high-precision automatic dicing saw (Disco Dad, no.3220) to keep the sides of the MLA clear in order to reduce vignetting and edge effect. The hybrid emission filter set consists of two filters: a glass interference filter (Chroma Technology, no. 535/50, 1 mm thickness) and a thin-film absorption filter (Edmund Optics, Wratten color filter no. 12, 0.1 mm thickness). We place the interference filter in front of the MLA and the thin-film absorption filter behind the MLA. This design reduces the maximum angle of incidence onto the interference filter to achieve better filtering whereas the remaining leakage is suppressed by the absorption filter. This design is further elaborated with the detailed spectral properties of the two emission filters under different angles of incidence in Section S2 and Fig. S2. All the optical elements and the CMOS sensor are held by a 3D-printed housing (designed in TinkerCAD, printed on Formlabs Form 2, black resin, no. RS-F2-04). The assembled imaging module is mounted to the CMOS PCB by four mini set screws and hex nuts (Thorlabs, no. HW-KIT3). The assembled CM² V2 prototype has a calibrated de-magnification of ~1.7× which results in an effective pixel size of 4.15 µm. The CM² V2 prototype does not require precise alignment of the optics. The field varying PSFs are experimentally calibrated and later computationally analyzed by our 3D-LSV model (see Section 2.2).

The illumination module consists of four freeform LED-illumination units held together by a 3D-printed dome-shaped base plate, which also blocks the ambient light (more details of the design are provided in Section S1). Each freeform LED-illumination unit contains the following parts: a surface mounted LED (Lumileds, LXML-PB01-0040), a 3D-printed freeform collimator (printed on Formlabs Form 2, clear resin, no. RS-F2-GPCL-04), a glass excitation filter (Chroma Technology, no. 470/40, 4 mm), and a 3D-printed LED housing (printed on Formlabs Form 2, black resin, no. RS-F2-GPBK-04). To achieve better surface quality, we post-process the 3D-printed collimator by the following procedure. A thin layer of clear resin (diluted 5 times with isopropyl alcohol) is applied to the outer parabolic surface of the collimator and cured under a UV lamp. The four illumination units are wired sequentially and connected to an LED driver (LED-dynamics Inc., 3021-D-E-350, 350 mA). The four LED units are placed symmetrically around the imaging path with a lateral offset of ~6.7 mm from the optical axis and tilted by ~45 degrees. The positions and orientations of the four illumination units are modeled and optimized in Zemax to achieve the maximum light delivery efficiency and overall excitation uniformity at the imaging plane (see more details in Section S1). After the CM² V2 is fully assembled, the excitation is validated on a green fluorescence calibration slide.
(Thorlabs, no. FSK2). The total excitation power across the designed 8-mm region is up-to 75 mW (at maximum driving current of 350 mA), measured by a power meter (Thorlabs no.PM121D). The illuminator is turned on at 300 mA continuously for 1 hour and no overheating issues were observed.

### 4.2 Sparse PSF calibration process

The spatially varying PSFs of the assembled \( \text{CM}^2 \) V2 are experimentally calibrated. The calibration process collects the 3D-LSV PSFs at a set of 3D sparse locations within the targeted imaging volume. To perform the calibration, we first build a point source setup consisting of two parts: a point source and a 3-axis high-precision automatic scanning stage. The point source is built by first stacking multiple layers of thin diffusing films (Parafilm) on top of a bright green LED source (Thorlabs, M530L4), whose spectrum approximately matches with the green fluorescence. A 5-\( \mu \)m mounted pinhole (Thorlabs, P5D, stainless steel) is placed immediately after the diffusing films. The multi-layer diffusing film homogenizes the LED illumination before entering the pinhole. The resulting point source provides a large divergence angle (>60 degrees), i.e. a large illumination NA, which is approximated as a point source. The angular intensity distribution from this point source is characterized in Fig. S6b. The point source is mounted on a 3-axis scanning stage (Thorlabs, MT3Z8) that is automatically controlled by a custom MATLAB script. To calibrate the 3D LSV PSFs, the point source is scanned across a sparse grid. The 3D grid spans [-4 mm to 4 mm] along both lateral dimensions and [-500 \( \mu \)m to 500 \( \mu \)m] along the axial dimension. The scanning step size is 1 mm laterally and 100 \( \mu \)m axially, respectively. In total, we collect PSFs on a \( 9 \times 9 \times 11 \) grid and 891 measurements. The MATLAB script controls both the stage scanning and provides the triggers for the \( \text{CM}^2 \) V2 image acquisition. To further account for the non-uniform angular profile of the point source, the MATLAB script adaptively adjusts the exposure times at different scanning locations. This ensures all the measured PSFs are not saturated or too dark. The exposure time is recorded to later normalize the PSF measurements before the 3D-LSV modeling. The whole calibration process takes about 2 hours to complete.

### 4.3 The 3D-LSV model

Our 3D-LSV decomposition and interpolation algorithm is performed in the following steps. A detailed diagram is shown in Fig. S3.

1. The experimentally calibrated PSF set is normalized by the exposure time. Each raw PSF measurement contains 2076 \( \times \) 3088 pixels.
2. To enable memory efficient decomposition, we crop 9 small patches, each having 160 \( \times \) 160 pixels, around the \( 3 \times 3 \) foci from each PSF image and remove the surrounding dark regions. Each cropped patch is centered around the chief ray at the image plane from the point source passing through the corresponding microlens.
3. We first estimate the axial shearing slope under each microlens based on the measured on-axis PSF stack. Next, we use the subpixel translation function \( \text{imtranslate} \) to compensate for the depth-dependent lateral shift for each cropped PSF. This compact, cropped PSF stack enables efficient SVD decomposition to model the 3D-LSV PSFs.
4. Each set of 9 registered PSF patches are re-combined to form the \( 3 \times 3 \) array foci. The entire 891 preprocessed PSFs are reshaped into a \( 480^2 \times 891 \) “calibration matrix”, where each column vector represents a single calibrated PSF.
(5) The calibration matrix is decomposed using the singular value decomposition (SVD) and truncated to the leading 64 terms (having the largest singular values) to construct the basis PSF matrix $H$ (size $480^2 \times 64$) and the coefficient matrix $M$ (size $64 \times 891$), as described by Eq. (1). Each basis PSF are found by reshaping each volume vector in $H$ back to a $480 \times 480$ image. The coefficient volume of each basis PSF is found by reshaping each row vector in $M$ back to a $9 \times 9 \times 11$ matrix (i.e. the sparse calibration grid). The result of this low-rank decomposition is visualized in Figs. 3c and S4.

(6) We interpolate all the coefficient volumes onto a finer $1928 \times 1928 \times 80$ grid using the 3D bilinear interpolation method, corresponding to the $8 \times 8 \times 0.8$ mm$^3$ volume with 4.15-µm lateral and 10-µm axial sampling steps. The result of this 3D interpolation is visualized in Fig. 3c.

(7) To construct the CM$^2$ measurement in its original format containing $2076 \times 3088$ pixels, the cropped PSF patches are placed back to their expected pixel locations. The locations are determined based on the chief ray location at the image plane from the point source location passing through the corresponding micro lens, and the estimated axial sheering in step #3.

(8) Each CM$^2$ measurement is simulated based on Eq. (2) on each synthetic object volume. The synthetic object volume generation procedure is detailed in Section 4.4.

4.4 Synthetic training data generation

Using the proposed 3D-LSV model, we generate a large-scale data set for training and testing the proposed CM$^2$Net. In this section, we provide details about the synthetic data generation, including the construction of synthetic 3D objects, data augmentation, and data preprocessing.

Each simulated CM$^2$ measurement is computed based on Eq. (2) from a synthetic volume. The FOV of each synthetic volume takes a random value that is drawn from a uniform random distribution between 6.5 mm and 7.5 mm, $U[6.5 \text{ mm}, 7.5 \text{ mm}]$, which is around our targeted FOV. Since the degree of view-multiplexing in the CM$^2$ measurement is directly determined by the FOV$^{28}$, this large ~7-mm FOV ensures the trained CM$^2$Net is generalizable to an arbitrary imaging FOV within the device's fundamental capacity. For FOV < 2.7 mm, no view-multiplexing is present and the CM$^2$ measurement consists of 3 × 3 non-overlapping views of the 3D object. As the FOV increases, the amount of overlap between the neighboring views increases approximately quadratically. At the largest FOV = 7.5 mm, approximately 64% of overlap is present between the neighboring views. The depth range of the synthetic objects is fixed at 800 µm, which is our targeted reconstruction range. The synthetic volumes are sampled at 4.15 µm laterally (matching the effective pixel size of the CM$^2$ V2) and 10 µm axially (10× higher sampling than the physical scanning step size).

We randomly place spherical emitters into the volumes by the following steps. Due to the large sampling grid size ($4.15 \times 4.15 \times 10$ µm) in the CM$^2$ reconstruction as compared to typical fluorescence emitter size (~10 µm), we first generate each ground-truth emitter on a $5 \times$ finer grid ($0.83 \times 0.83 \times 2$ µm). To generate a ground-truth volume having the same grid size as the CM$^2$ reconstruction, we perform down-sampling by $5 \times 5 \times 5$ average binning. We normalize the intensity for each emitter with its cross-sectional area. The size of the emitters follows a uniform random distribution $U[8 \mu m, 20 \mu m]$, which is designed to approximately match the typical size of neuronal cell bodies. To further vary the intensities of the emitters at a given size, a random scaling factor is introduced that is drawn from a uniform distribution $U[0.8, 1.2]$. This scaling factor is chosen to approximately match with the typical contrast obtained by one-photon fluorescence microscopes on common Calcium indicators (the GCaMP family). The emitter density in each volume follows a uniform random distribution $U[10, 100]$ (number of emitters / mm$^3$), which simulates different fluorescent labeling densities.

We first generate noise-free CM$^2$ measurements from the synthetic volumes using
the 3D-LSV model. We then augment the data with realistic levels of mixed Gaussian and Poisson noise to mitigate the distributional differences between the simulated and experimental data. The parameters for the additive Gaussian noise (normalized mean = 0.048, standard deviation = 0.017) are estimated by taking multiple dark measurements with the assembled CM² V2 device using the same acquisition parameters as the real experiments (30 ms exposure time, 40 dB gain). The Poisson noise is added by estimating the expected photon budget (~500 peak number of photons, and a unit effective gain) in typical widefield one-photon imaging.

We also simulate CM² measurements using the depth-wise LSI model following the same general procedure and synthetic objects as above.

Before feeding the synthetic CM² measurements to CM²Net for training and testing, the following preprocessing steps are taken. First, we crop each of 9 overlapped views from the raw measurements based on each chief ray location at the image plane of each microlens from the emitters at the nominal focal plane, which is set as the middle slice of the imaging volume in the simulation. Each cropped view has a size of 1920 × 1920 pixels. Next, we directly stack the 9 cropped views to form a 1920 × 1920 × 9 multi-channel input to CM²Net, as illustrated in Fig. 5.

To train the view demixing-net module, we further generate the 9 ground-truth non-overlapping views from each microlens using the same 3D-LSV forward model but with the single-microlens PSF. Each single-microlens PSF can be easily extracted since it is isolated from the rest. In addition, our 3D-LSV model is directly applicable to simulate single-microlens imaging. The network training is performed on randomly cropped small patches (320 × 320 pixels). In total, we generated ~9700 training patches taken from 270 synthetic objects. The training data generation takes ~8 hours on Boston University Shared Computing Cluster (Linux, Intel Xeon E5-2680v4, 128 GB). An independent testing data set is generated following the same pipeline. The testing set contains ~180 simulated CM² measurements.

4.5 Implementation of CM²Net

The high-level design of the CM²Net is shown in Fig. 5. It contains three sub-networks, including the view demixing-net, view-synthesis-net, and lightfield-refocusing enhancement-net. In the following, we describe the details of the network implementation and its training and testing procedure. Additional details are provided in Section S4.

The three sub-networks all use the same residual network structure and only differ in the input/output dimensions. Each sub-network contains 16 residual blocks, each consists of two consecutive 2D-convolution layers with 64 filters of kernel size 3, two batch normalization layers and a Parametric ReLU layer. The numbers of input and output channels, denoted by (a, b) for the demixing-net, view-synthesis-net, and enhancement-net are (9, 9), (9, 80), and (32, 80), respectively, as detailed below.

The CM²Net takes a stack of 9 cropped views from a CM² raw measurement as the input. In the training phase, the input data dimension to the first demixing-net is 320 × 320 × 9, where the third dimension denotes the number of channels. The output size from the demixing-net remains the same. The non-learnable lightfield refocusing module applies a simple “shift-and-add” refocusing operation on the demixed 9 views. The amount of shift is chosen to be [-18: +17] pixels (+ means shifting towards the center view, - means the opposite), which ensures that the refocused stack consisting of 36 planes will cover the targeted 800-µm depth range. Note that this refocusing algorithm generates artifacts around the image boundaries. Therefore, we discard the outmost 32 pixels in both lateral dimensions, resulting in a 256 × 256 × 36 refocused volume. The enhancement-net is trained to first resample the refocused volume onto an axially finer sampling grid (256 × 256 × 80) matching the ground-truth volume, and then enhance the
3D reconstruction. The resampling is performed by a 2D convolution layer of kernel size 3 with 80 channels, which increases the depths from 36 to 80. To perform the view-synthesis, similarly only the central 256 × 256 × 9 region is extracted from the demixed views and fed into the view-synthesis-net. The output dimension from the view-synthesis-net is 256 × 256 × 80, the same as the ground-truth volume.

In the inference phase, we directly feed the entire full-FOV measurement to perform a single-pass 3D reconstruction, which bypasses the stitching artifacts suffered by patch-wise reconstructions.

The loss function for CM²Net training combines a demixing loss and a reconstruction loss: loss = α₁l_{demix} + α₂l_{rec}. For both loss components, we use the binary cross entropy (BCE): BCE(y, ŷ) = ∑ᵢyᵢ log(ŷᵢ) + (1 − yᵢ) log (1 − ŷᵢ), where the summation over all the voxels indexed by i, and y and ŷ denote the ground-truth and reconstructed intensity, respectively. We choose BCE because of its robustness to reconstruct sparse objects. The weights of the two loss functions (α₁, α₂) are set to be (1, 1) after performing hyperparameter tuning, which concluded that the demixing and reconstruction losses have equal importance in the CM²Net framework.

The CM²Net is implemented in Python 3.7 with TensorFlow 2.3. The multiple sub-networks in CM²Net are trained together in an “end-to-end” fashion on an Nvidia P100 GPU (16 GB) with a batch size of 2. We use the Adam optimizer with an adaptive learning rate schedule to optimize the parameters in the CM²Net. The initial learning rate is 10⁻⁴ and automatically decreases by a factor of 0.9 after the loss on a small validation set (~400 patches) plateaus for 2 consecutive epochs. The training takes ~48 hours to complete. After training, we test the inference (3D reconstruction) time on a local computer with an Nvidia RTX 2070 GPU (8 GB). The inference time for reconstructing a 7.5-mm FOV, 800-µm DOF volume (input size: 1920 × 1920 × 9, output size: 1856 × 1856 × 80) is ~3.6 seconds.

4.6 Preparation of phantom objects and table-top widefield measurements

Fluorescent 3D objects are prepared according to the following protocol. Green fluorescent particles (Thermo Fisher Scientific, Fluoro-Max Dry Fluorescent Particles, 10-µm and 15-µm) are added to ~1 mL of clear resin (Formlabs, no. RS-F2-GPCL-04). The mixture is then diluted and moved onto a standard 3 × 1 inch microscope slide (Thermo Fisher Scientific, no. 125493) and curved under a UV lamp for ~30 minutes. We use a micropipette (Thermo Fisher Scientific, Adjustable Volume Pipette, 10-100 µL, no. FBE00100) to control the transferred volume of mixture (~40 µL) so that the object is ~800 µm thick and 7-mm wide.

To verify the 3D reconstructions from CM²Net, we experimentally collect axial focal stacks (z-stack) on a commercial table-top epi-fluorescence microscope (Nikon TE2000-U) with GFP filter sets (Thorlabs, no. MDF-GFP) and a scientific CMOS camera (PCO Imaging, Pco.edge 5.5). To acquire the full-FOV widefield measurements, we use a low-magnification objective lens (Nikon, CFI Plan Apo Lambda 2×, 0.1 NA) with 50-µm axial step size across the 800-µm depth range. To acquire a high-resolution axial scan at zoom-in regions, we use a high-magnification objective lens (Nikon, CFI Plan Achromat 20×, 0.4 NA) with 25-µm axial step size across the 800-µm depth range.

4.7 Preprocessing of the experimental data

We take the following steps to preprocess experimental measurements before feeding them to CM²Net for 3D reconstruction. The goal is to match the intensity
distribution between the simulated and the experimental data so that our simulator-trained CM\textsuperscript{2}Net can generalize well to real measurements. The main discrepancy between the measured and simulated images is the low-frequency fluorescent background due to the reflection from the microscope slide. To remove the background and match the intensity statistics, we preprocess the experimental measurements with histogram matching. The reference histogram is estimated from the entire training dataset. The histogram matching builds a monotonic mapping from the experimental data to the simulated data. A comparison between the histograms of the simulated data, the experimental data with and without histogram matching are provided in Fig. S7. We find that other commonly used background removal methods (such as thresholding, morphological opening, etc.) do not generalize well on real experimental measurements. Another preprocess step is to adjust the centers of the cropped 9 views to compensate for the small displacement of the chief ray positions between the simulation and experiments. The adjustment is done by manually aligning a few on-axis particles in each view.

4.8 Performance evaluation metrics

We use particle-wise detection metrics, including recall, precision and F1-score to quantitatively evaluate the CM\textsuperscript{2}Net 3D reconstruction. Here we describe the details on the computations of the three metrics using MATLAB language. Starting from the reconstructed volumes from the CM\textsuperscript{2}Net, we first detect all the recovered particles within the volume. The detection is done by binarizing the recovered 3D volume with a global optimal thresholding (\textit{imbinarize}) and then extracting the locations and sizes of connected 3D components (recovered particles) from the binarized volume (bwconncomp, regionprops3). We then compute a distance matrix by calculating the Euclidean distance between every recovered and ground-truth particle and solve a linear assignment problem based on the distance matrix to assign the recovered particles to the corresponding ground truth (\textit{matchpairs}).

If the Euclidean distance between the recovered and the matched ground-truth particles is larger than a pre-defined distance threshold (i.e. 2\(\times\) the particle size in our simulation), we count it as a False Negative (FN), meaning that the CM\textsuperscript{2}Net fails to correctly reconstruct the particle. If a recovered particle does not find a match in the ground truth, we count it as a False Positive (FP). A True Positive (TP) is when the distance between the recovered and the matched ground-truth particles is smaller than the distance threshold. Lastly, the recall, precision and F1-score are computed as follows:

\[
\text{Recall} = \frac{\#TP}{\#TP + \#FN},
\]

\[
\text{Precision} = \frac{\#TP}{\#TP + \#FP},
\]

\[
F1 = \frac{2}{\text{Recall}^{-1} + \text{Precision}^{-1}}.
\]

Recall and precision quantify the detection performance in two complementary aspects. Recall quantifies the CM\textsuperscript{2}Net’s sensitivity by the fraction of emitters it correctly reconstructed out of the total in the ground-truth volume. Precision measures the fraction of emitters it correctly reconstructed out of the total reconstructed emitters. F1-score is a metric combining recall and precision. The global threshold for binarizing the recovered volume is set by maximizing the F1-score on the evaluation set\textsuperscript{48}. 

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The metric maps reported in Sections 2.6 and 2.7 are computed by the metric value on non-overlapping patches (250 µm × 250 µm in Section 2.6 and 500 µm × 500 µm in Section 2.7). When quantifying the metrics on experimental data (Sections 2.7 and 2.8), we first co-register the CM²Net reconstruction and the widefield measurement by manually selecting a few matched feature points in the central FOV. We then extract particles by binarizing both the widefield measurement and the CM²Net reconstruction. Next, we follow the same evaluation pipeline as above. Since the amount of image distortion suffered by the widefield and CM² measurements are different, good registration can only be achieved around the central region, while particle pairs at the peripheral region are expected to have a larger amount of separations. Therefore, the distance threshold we choose for the experimental data is ~5× the particle diameter, which is larger than the one used for the simulation (2× the particle diameter). The binarized MIP image pairs are shown in Figs. S9b and S11b for the 10-µm and mixed-size bead phantom, respectively. Moreover, since the particles in the experiment are not uniformly distributed, we observe NaN values (both the widefield measurement and the reconstruction in this image patch are empty) when perform quantification for the experimental data. For fair evaluation, we exclude the NaN values when calculating the mean and standard deviation. When making the metric maps, we linearly interpolate the pixels having NaN values, and applies a binary mask to indicate the sample region.

In Fig. 8, the line plots show the quantitative metrics under different conditions. Each point and the associated error bar represent the mean and standard deviation, respectively. The testing data set contains in-total 180 random volumes and ~5×10⁵ emitters. Each point in Fig. 8b is computed on ~20 volumes for each range of emitter density. Each point in Figs. 8a, c and d is computed on emitters for each range (labeled on the horizontal axis) of lateral location, depth, and emitter size.
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Author contributions
Y.X and L.T. conceived the idea. Y.X. prototyped the hardware platform and algorithm, and conducted the experiments. Q.Y. helped with the reconstruction algorithms. G.H. and K.G helped with the experiments and system calibration. All authors participated in the writing of the paper.

Conflict of interest
The authors declare no competing interests.

Data availability
The CM²Net implementation and pretrained model reported in this work are available at: https://github.com/bu-cisl/Computational-Miniature-Mesoscope-CM2
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Supplementary Information

Computational Miniature Mesoscope V2: A deep learning-augmented miniaturized microscope for single-shot 3D high-resolution fluorescence imaging

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This document provides supplementary information for the manuscript “Computational Miniature Mesoscope V2: A deep learning-augmented miniaturized microscope for single-shot 3D high-resolution fluorescence imaging”. We provide more details on the CM² V2 hardware design and prototype assembly. We further explain the detailed processing procedure of the proposed 3D LSV decomposition model and elaborate on the implementations of CM²Net. Lastly, we provide additional analysis for the experimental results.
Figure S1: Additional details on the CM² V2 design and the prototype. (a) Top and side views of the 3D printed housing (in gray) that holds the optics (not drawn) and the sensor (in red and green). (b) Mechanical design of the freeform collimator and the ray tracing diagram with the interference-excitation filter from Zemax. (c) Photos of the assembled illumination units, the 4-LED array, and the CM² V2 prototype. Each illumination unit hosts a surface mounted LED source, the 3D printed freeform illuminator, and the excitation filter.

Section S1. Additional Details on the CM² V2 Design and Prototype

In this section, we provide additional details on the CM² V2 design and prototype assembly. Figure S1a shows the top and side views of the 3D printed MLA housing and how it is mounted onto the CMOS PCB board (IDS Imaging, board-level IMX178). The red region indicates the sensor region of the CMOS. The green regions are the PCB that host extra electronics. In Fig. S1b, we provide the exact mechanical design and the ray tracing diagram of the freeform illuminator. The ray tracing in Zemax has incorporated the LED source spectrum and the incidence-dependent transmittance profiles of the interference-excitation filter from the manufacturer (Chroma Technology). The ray tracing result shows the freeform illuminator efficiently refracts and reflects light from the LED source and allow them to pass through the interference coating. The small divergence of outcoming light is because the LED source has an emitting area of 1 mm². The assembled illuminator units (including 3D printed housing, LED source, freeform collimator, and the excitation filter) are shown in Fig. S1c Left. The four units are tilted by ~45 degree and installed into the LED base plate. Figure S1c Middle and Right show the wired illumination array and fully assembled CM² V2 prototype, respectively.
Section S2. Spectral Properties and the Design of Hybrid Emission Filter Set

The non-normal incidence of uncollimated light in CM² V1 results in a substantial wavelength shift of the transmitted spectrum of the emission filter. In Fig S2a, we plot the spectral profiles of the LED source (blue solid region), green fluorophore emission (green solid region), excitation filter (red curve), emission filter at 0, 20, 40, 60-degree incidence (yellow, purple, green, and blue curve, respectively), and the absorption filter (black curve). The transmittance window of the interference-based emission filter shifts to shorter wavelength with a degraded profile (the oscillating curves) at non-normal incidence. It has a wide spectral overlay with the excitation spectrum, results in a background fluorescence in CM² V1 measurements. By adding an incidence-independent absorption filter, whose long-pass spectral profile is plotted in Fig. S2a (black curve). Experimentally, we find that adding the absorption filter to the imaging path provides better suppression of background fluorescence at the cost of slightly reduced transmittance at emission wavelength. Figure. 2e in the main article has clearly shown that the hybrid emission filter provides much enhanced image contrast and reduced the background haziness by ~5 times.
Figure S3: Procedure diagram of our 3D-LSV model. Local small PSF patches are first cropped from the calibrated PSF measurements based on the chief ray locations. The cropped PSF patches are registered based on the estimated axial sheering and re-grouped into array PSFs. Then we perform TSVD on the registered array PSFs to obtain basis array PSFs and their coefficients at a sparse grid of calibrated locations. The coefficients are then 3D interpolated to the entire imaging volume with 3D bilinear interpolation. Next, the basis PSFs patches are placed back to their original locations. Lastly, the CM² measurement is computed by $k$ weighted 2D convolutions between the object volume and the basis PSFs, followed by a summation along the axial dimension $z$.

More examples of basis PSF and interpolated coefficient volumes

Figure S4: More examples of the decomposed basis PSFs and the interpolated coefficient volumes.
Section S3. Additional details on the 3D Linear Shift Variant (LSV) model

In this section we provide additional details of the 3D-LSV decomposition and interpolation pipeline and provide additional visualizations of the basis PSFs and the interpolated coefficient volumes. Figure S3 shows a diagram of the 3D-LSV decomposition and interpolation process. The experimentally calibrated PSFs (2076 × 3088 pixels) are first normalized by the exposure time recorded in the calibration process. Next, small PSF patches (160 × 160 pixels) are extracted from the PSF measurements based on the chief ray locations. Due to the finite conjugate imaging geometry of the CM² system, the PSF at a different depth exhibits a different amount of lateral shift that is approximately linearly increases with the depth (i.e. axial sheering). To enable efficient PSF decomposition, we estimate the amount of axial sheering based on the on-axis PSF stack and then align the PSF patches with the in-focus PSF by numerically “undo” lateral shift. The 9 aligned PSF patches are regrouped into a 3 × 3 foci array (480 × 480 pixels). Next, the 891 calibrated array PSFs are decomposed using the singular value decomposition (SVD) and truncated to the leading 64 terms. There are two products from this TSVD process: the 64 basis PSFs $H$ (480 × 480 pixels) and 64 coefficient volumes $M$ (9 × 9 × 11 voxels). To match our reconstruction sampling, the coefficient volumes are further interpolated onto a dense 1920 × 1920 × 80 grid using the 3D bilinear interpolation method. To construct the full-sized (2076 × 3088 pixels) CM² measurement, the basis PSF patches are then put back to their original pixel locations. The locations are determined by the chief ray locations and the estimated axial sheering. Lastly, the measurement is computed by $k$ weighted depth-wise 2D convolutions between the object volume and the basis PSFs, followed by a summation along the axial dimension $z$. In Fig. 3c of the main article, we visualize the first 5 basis PSFs and their coefficient volumes. Figure S4 shows the next 10 basis PSFs and their interpolated coefficient volumes.
Section S4. Implementation details of the CM² Net and its building block

In this section, we provide additional implementation details about the CM² Net. The three sub-networks in CM² Net: the demixing-net, enhancement-net, and the viewsynthesis-net, all have the same backbone structure of deep residual network. In our CM² Net implementation, all three residual networks have 16 consecutive residual-blocks. The detailed structure of a residual block is provided in Fig. S5. The input tensor first goes through a 2D convolution layer which has 64 convolution kernels with size 3 × 3. After the 2D convolution layer, the intermediate tensor is fed into a batch normalization layer, followed by a nonlinear activation layer using Parametric ReLU (PReLU) layer, a nonlinear function with learnable slope in the negative side of the axis. The tensor then goes through another pair of 2D convolution and batch normalization layers to further increase the receptive field. Lastly, the tensor is element-wise added to the input tensor of this block, which forms a residual connection.

Figure S5: Implementation details of CM² Net building block. The three sub-networks in the CM² Net share the same backbone structure of a deep residual network that connects 16 residual-blocks with sequential residual connections. Each residual-block consists of a 2D conv layer with batch normalization (BN), a Parametric ReLU nonlinearity, another 2D conv layer with BN, and element-wise addition.
Figure S6: Ablation study on the reconstruction module of the CM2Net using the ground-truth demixed views. (a) Yellow curve: recall for a retrained reconstruction module using the ground-truth demixed views as the input. The recall is close to 1 consistently for the entire 7-mm FOV range, showing the effectiveness and robustness of our reconstruction module design. Orange curve: recall for directly feeding the ground-truth demixed views to the reconstruction module of the pre-trained CM2Net. The recall remains >0.89 across the 7-mm FOV. Blue curve: recall for the trained CM2Net, which is equivalent to feeding the view-demixing-net demixed views to the reconstruction module of the pre-trained CM2Net. The decreased performance is attributed to the degraded view-demixing results. (b) Left: the intensity distribution of the point source used in our PSF calibration as measured by total intensity under a single microlens at different scanning positions. Right: the recall map of the CM2Net is visualized by binning onto the same pixel grid as the point source intensity map. Both maps exhibit much reduced values at the outer FOV regions, which suggests that the degraded recall at the outer FOV regions may be due to the rapid intensity decay of the point source used in the model. (c) Comparison of the view-demixing results between patches from the edge and central FOV. The central patch that achieves ~1 recall has higher SNR and shows nearly perfect view-demixing result. However, the view-demixing prediction on the patch from the edge FOV suffers from low SNR and missing particles.
Section S5. Ablation study on the reconstruction module of the CM\textsuperscript{2}Net

In this section, we perform an ablation study to analyze the potential reasons for the decrease of recall near the edges of the FOV. We first separate the CM\textsuperscript{2}Net into demixing network and the reconstruction network (consisting of the enhancement-net and the view-synthesis-net). First, we train another network with only the reconstruction network and take the ground-truth demixed views as the input. The recall for emitters at different lateral location are then evaluated on the testing set using the same method in Section 4.8. The reconstruction results achieve recalls close to 1 consistently for the entire 7-mm FOV range (Fig. S6a yellow curve), showing the effectiveness and robustness of our reconstruction network design. To further evaluate the trained CM\textsuperscript{2}Net, we quantify the recall of the reconstruction network in the pre-trained CM\textsuperscript{2}Net by directly inputting the ground-truth demixed views. The result (orange curve) shows a slight degradation as compared to the re-trained reconstruction network but still remain >0.89 across the 7-mm FOV. Both results are much higher than that from the CM\textsuperscript{2}Net predictions on the raw CM\textsuperscript{2} measurement (blue curve). This indicates that the CM\textsuperscript{2}Net’s reconstruction module provides superior performance, and the degraded performance at the outer FOV is due to imperfect view-demixing at these regions.

Upon visual inspection, we hypothesize that the training of the view-demixing network is impacted by the low light intensity and reduced SNR of the point source near the edge of the FOV. To show this, we calculate the intensity map of the point source under the central microlens in Fig. S6b and show that the point source’s intensity drops as much as ~85% near the edge as compared to the intensity at the central FOV. Moreover, we compare the point source intensity map and recall map on the same pixel grid. Both maps exhibit similar non-isotropic distributions and significant drops at outer FOV regions. Finally, we show comparisons between the ground-truth and the view-demixing-net predicted demixed views (1-mm\textsuperscript{2} patch) for both a central and an edge image patches in Fig. S6c. The view-demixing result for the central FOV is highly accurate; whereas the result near the edges suffer from a large amount of missing particles. We also visually observe that the SNR for the demixed views at the edge FOV are much worse than the central FOV. Overall, this ablation study shows that the combination of the view-synthesis-net and enhancement-net can achieve superior reconstruction performance and is robust to large SNR variations and high dynamic range of the measurements. We provide evidence that the low recall near the edge is possibly due to the unevenly distributed point source used in the PSF calibration.
Figure S7: Preprocessing on experimental CM² measurements with histogram matching. Preprocessed experimental measurements have a suppressed background and a better match with the intensity distribution of the simulated training data.

Section S6. Histogram preprocessing on experimental data

In this section, we provide additional details on the preprocessing of experimental CM² measurements. The goal of data preprocessing is to make the input measurement match closer with the 3D LSV simulated training dataset so that the simulator-trained CM²Net can generalize to experimental data. Experimental measurements have a slightly higher background floor (see Fig. S6) due to the reflection from the microscope slide, which is not accounted for in our simulation pipeline. We use a standard image processing algorithm called “histogram matching” to match the experimental and simulated data. The reference histogram is first computed from the entire training dataset. Then the histogram matching algorithm builds a monotonic intensity mapping that transforms from the distribution in experimental data to simulated data. Examples of an experimental measurement on an object of 10-µm fluorescent beads before and after histogram matching are shown in Fig. S6 where one can clearly see the background is much suppressed after the preprocessing. This can be further verified in their histograms. The distribution of preprocessed experimental data matches simulated data much better than raw experimental measurements.
Figure S8: Extra views in the 10-µm bead experimental measurement. The partial microlenses adjacent to the 3 × 3 microlens array generate extra views in the experimental measurement. The extra views contaminate the 9 central views, which results in increased false positives and mis-detections in the experiments.
Figure S9: Quantitative evaluation on 10 μm bead experimental data. (a) The recall, precision, and F1-score maps for the measurement in Fig. 9 of the main text (sample: 10-μm fluorescent beads in a cylindrical volume with 6.7-mm diameter and 0.5-mm depth). The 'x' label indicates the metric value is zero. The dashed circles show the expected boundary of the phantom object. (b) The overlay between the registered and binarized widefield measurement and the CM²Net reconstruction. Patch 1 (size: 1-mm²) is from the central FOV that achieves the highest F1 = 1.0. In region 1, the binarized map for widefield measurement match perfectly with the CM²Net reconstruction. Patch 2 is from the edge FOV that has a low precision but with perfect recall (i.e. all ground-truth particles are matched but with unmatched falsely reconstructed particles). Patch 3 is from the edge FOV and has a low recall but with perfect precision (all detected particles are matched but with unmatched ground-truth particles). Note that since the image wrapping is unavoidable in the real experimental data and the registration is based only on the central particle pairs, the particle pairs at the peripheral FOV are expected to exhibit slight separations.
Figure S10: Extra views in the mixed-size bead experimental measurement. Compared to the mono-10µm bead experiment, the reduced FOV (6.7mm to 6.5mm) reduces the level of contaminations from the extra views, which results in decreased false positives and higher precision in the reconstruction.
Figure S11: Quantitative evaluation on mixed-size bead experimental data. (a) The recall, precision, and F1-score maps for the object in Fig. 10 of the main text (sample: 10-µm and 15-µm mixed fluorescent beads in a cylindrical volume with 6.5-mm diameter and 0.8-mm depth). The x labels indicate the metric value is zero and the dashed circles show the expect 6.5-mm diameter region of the phantom object. (b) The overlay of the registered and binarized widefield measurement and CM²Net reconstruction. Patch 1, 2, and 3 (size: 1-mm²) show example regions of having high F1-score, low recall, and low precision, respectively.

Section S7. Recall, precision, and F1-score maps in phantom experiments

In this section, we report the details of recall, precision, and F1-score evaluations on the two experiments in the main article. The recall and precision maps are computed on non-overlapping patches (500 µm × 500 µm) across the entire FOV.

For the 10 µm beads phantom in Fig. 9, the recall and precision maps are shown in Fig. S9a. The CM²Net reconstruction achieves a recall ~0.78 and precision ~0.80. In comparison, the recall and precision in simulation at the corresponding density range is ~0.83 and ~0.97, respectively (Fig. 8b). This shows that the simulator-trained CM2Net degrades slightly on experiments with ~5% higher false positive rate and ~17% higher mis-detection rate at this imaging condition. We attribute the reduced performance to the undesired extra views in the experimental measurements due to an extra column of partial microlenses adjacent to the main 3 × 3 microlens array (see Fig. S8).

For the mixed-size beads phantom in Fig. 10, the recall and precision maps are shown in Fig.S11a. The CM²Net achieves averaged recall ~0.73 and precision ~0.84 across the entire 6.5-mm FOV. As compared to the mono-10µm bead experiment, we hypothesize that the decreased recall is attributed to the greater intensity and SNR variations caused by bead size variation in the measurement. The increased precision is due to the reduced FOV and less contaminations from the extra views (see Fig. S10).

For better visualization, we further show the overlays of the registered and paired binarized maps between the widefield measurement and the CM²Net reconstruction.
We zoom-in on three patches (size: 1 mm$^2$) in the metric maps and the corresponding overlay map. Patch 1 from the central FOV achieves the highest F1 score 1.0. The patch with index 2 and 3 is extracted from the edge FOV that either has a low precision with perfect recall (all ground truth particles are matched but with redundant detection) or a low recall with perfect precision (all detected particles are matched but failed to detect some ground truth particles). Patch 2 is from the edge FOV that has a low precision but with perfect recall (i.e. all ground-truth particles are matched but with unmatched falsely reconstructed particles). Patch 3 is from the edge FOV and has a low recall but with perfect precision (all detected particles are matched but with unmatched ground-truth particles). Note that since the image wrapping is unavoidable in the real experimental data and the registration is based only on the central particle pairs, the particle pairs at the peripheral FOV are expected to exhibit slight separations. For direct visualization, we enlarge the corresponding overlap maps in Figs. S9b and S11b. We show in patch 1, the particles detected from the widefield measurement match perfectly with the particles reconstructed by the CM$^2$Net. In patches 2 and 3, we show unpaired particle detected from either the CM$^2$Net reconstruction (low precision) or the widefield measurement (low recall), which are consistent with our metric map, which validates the accuracy of our evaluation procedure.