# Compact Quantum-Dot Microbeads with Sub-Nanometer Emission Linewidth

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Fluorescent microbeads are widely used for applications in life sciences and medical diagnosis. The spectral contrast and sharpness of photoluminescence are critical in the utilities of microbeads for imaging and multiplexing. Here, microbeads capable of generating single-peak laser emission with a sub-nanometer linewidth are demonstrated. The microbeads are made of quantum dots that are tightly packed and crosslinked via ligand exchange for high optical gain and refractive index as well as material stability. Bright single-mode lasing with no photobleaching is achieved with particle diameters as small as 1.5  $\mu$ m in the air. Sub-nm lasing emission is maintained even inside high-index surroundings, such as organic solvents and biological tissues. Feasibility of intracellular tagging and multi-color imaging in vivo is demonstrated.

## 1. Introduction

Microbeads are a widely used platform for molecular and cellular analyses.<sup>[1]</sup> They are typically made of synthetic polymers and doped with fluorophores for color encoding. Their surface areas are adequate for capturing target molecules with high dynamic ranges relevant to biological systems. The micron sizes similar to those of cells or subcellular organelles are readily compatible with standard optical instruments, such as optical microscopy and flow cytometry, for readout. One popular utility of fluorescent microbeads is multiplex assays for immunoassay and drug development. Microbeads with different colors are conjugated with different antibodies or oligonucleotides against specific target proteins, DNAs, or RNAs, and allow spectrally multiplexed quantification of the analytes using imaging or high-throughput flow cytometry.<sup>[2]</sup> Fluorescent microbeads are useful in imaging and tracking in vitro and in vivo. In these and other applications, spectral brightness and photostability are always desired, and multicolor is the key to multiplexing.<sup>[3]</sup>

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Recently, there has been growing interests in using microbeads in a lasing or stimulated emission regime.<sup>[4,5]</sup> In this regime, the output emission of microbeads is dominated by amplified, one to several resonant cavity modes, each with a sharp spectral linewidth. As such, spectral brightness and multiplexing capability can be greatly enhanced. To reach lasing threshold, high-quality cavity resonance and sufficient optical gain are required. The optical pump fluence for lasing threshold critically depends on the refractive index (n) and diameter of microbeads. In earlier works,<sup>[4,5]</sup> standard polystyrene microbeads doped with fluo-

rescent dyes have shown to generate a few spectral peaks corresponding to different whispering gallery modes (WGM). With n = 1.59 for polystyrene, lasing was possible with sizes larger than 8 µm in water and 50 µm in biological tissues ( $n \approx 1.45$ ).<sup>[6]</sup> More recently, smaller laser particles with sizes of 0.5-2 µm have been developed using high-index high-gain bulk semiconductor crystals in various forms such as rods, plates, disks, and cubes.<sup>[7-10]</sup> However, they require relatively complex cleanroom nanofabrication processes and microcrystal growth at low throughput, and their non-spherical shapes could be less ideal than spherical microbeads in particle handling and for certain applications. A single mode perovskite sphere laser with submicron size was developed by using chemical vapor deposition method, but high solubility of perovskite in biological environment is not desirable for bio-application.<sup>[11]</sup> Colloidal semiconductor quantum dots (QDs) are attractive materials with high gain coefficients, refractive index, and excellent photostability<sup>[12-14]</sup> for building microbeads via self-assembly.<sup>[15-17]</sup> However, self-assembled microbeads lack in physical and chemical stability and have lower gain and refractive index than bulk semiconductors. Most of the spherical micro-lasers that have been demonstrated to date were larger than 5 µm in diameter and tended to oscillate with multiple spectral peaks associated with multiple cavity modes supported within the gain bandwidth of the material.<sup>[18–20]</sup>

Here, we demonstrate a novel type of microbead that carries most of the desirable characteristics of conventional polymeric microbeads but offers a uniquely appealing feature: single emission peak with sub-nanometer linewidth. We used colloidal QDs with monodentate ligands as building blocks of microbeads. A key innovation we introduce is substituting the existing monodentate ligands with short bidentate ligands, resulting in densely packed, crosslinked QDs in spherical shapes. The ligand exchange enhances the refractive index, www.advancedsciencenews.com



optical gain, and physical and chemical stability of the microbeads. We achieve lasing of microbeads as small as 1.5  $\mu$ m in size and demonstrate bright, stable, narrowband emission from microbeads in various environments including water, organic solvents, high-index polymers, and live cells and animals. Multicolor imaging with proposed microbeads shows enhanced spectral density and color-multiplexing capability compared to conventional fluorescence imaging.

## 2. Results

# 2.1. Fabrication of Compact QD Microbeads Using Bidentate Ligand Exchange

To overcome the shortcomings of previous approaches, we designed a new fabrication strategy using ligand exchange.<sup>[21,22]</sup> For stability during synthesis and storage in colloidal solution and surface passivation, QDs are commonly encapsulated with organic ligands such as oleate, oleylamine, and octadecylamine (ODA).<sup>[23]</sup> We hypothesized that replacing the monodentate ligands by shorter bidentate ligands could allow higher density packing of QDs and, thereby, enhance the optical and physical properties of microbeads.<sup>[24,25]</sup> Figure 1a illustrates the fabrication steps to synthesize self-assembled QD microbeads followed by ligand exchange. We used CdSe/ZnS core shell QDs with an average size of 8.2 nm, which are passivated by ODA with a length of ≈2.4 nm. QD microdroplets are produced in a glass microfluidic chip using an oil-in-water emulsion method.<sup>[26]</sup> Then, the solvent is evaporated.<sup>[17]</sup> The QD microdroplet size in the oil phase is reduced by 72% in diameter after solvent drying (Figure S1, Supporting Information). The fully dry QD microbeads are transferred to a solution containing bidentate ligands for solid-state ligand exchange. Prior to the ligand exchange, QDs in the microbeads are held together by van der Waals force (Figure 1b). After replacing the monodentate by short bidentate ligands, the QDs are brought closer and crosslinked (Figure 1c). Conventional non-crosslinked QD microbeads immediately dissolved upon adding toluene, but crosslinked QD microbeads showed excellent chemical stability in toluene (Video S1, Supporting Information).

1,8-diaminooctane (DAO) produced the best results among several bidentate ligands tested. Shorter amine ligand (1,2-ethylenediamine) and different functional groups (1,8-octanedioic acid, 1,8-octanediphosphonic acid, and 1,8-octanedithiol) with similar lengths resulted in reduced photoluminescence (PL) quantum yield (Table S1, Supporting Information).

# 2.2. Optical Properties of QD Thin Films with Mono- and Bi-Dentate Ligands

To investigate the effects of ligand exchange on optical properties, we prepared QDs as thin films. The typical thickness of ODA-QD films was measured to be 86 nm. After ligand exchange to DAO-QD, the thickness was reduced to 63 nm, ≈27% reduction in thickness. Figure 2a showed the PL spectra of ODA-QD and DAO-QD films, both with a full-width-at-halfmaximum (FWHM) of 30 nm. The PL peak wavelength of ODA-OD was 635.7 nm, which is shifted from the 632.0 nm PL peak of colloidal QDs in octane solution (Figure S2, Supporting Information). This red spectral shift is due to quantum coupling of QDs in the film. The PL peak of DAO-QD was 636.1 nm. This further spectral shift indicates increased inter-QD coupling due to the reduced inter-QD distance.<sup>[27]</sup> Figure 2b shows transient PL decay curves. They are best fit with bi-exponential curves with fast and slow time constants, which correspond to multi-exciton and single-exciton decays, respectively.<sup>[28]</sup> For ODA-QD samples, the time constants were 4.57 ( $A_1 = 0.56$ ) and 13.6 ns ( $A_2 = 0.41$ ). DAO-QD samples showed 3.39 ( $A_1 = 0.65$ ) and 10.7 ns ( $A_2 = 0.28$ ). The amplitude weighted average lifetimes are then 8.39 ns for ODA-QD and 5.59 ns for DAO-QD.



**Figure 1.** Fabrication of QD microbeads. a) Fabrication workflow consisting of four major steps: microdroplet generation, drying, transfer, and ligand exchange. b) ODA-QD aggregates in a microbead prior to ligand exchange. c) DAO-QD in the microbead after ligand exchange. The densely packed, crosslinked final form offers improved chemical, mechanical, and optical properties.





**Figure 2.** Photophysical properties of QD thin films before and after ligand exchange. a) Measured PL spectra. b) Transient PL decay. Dash lines, biexponential fitting curves. c) Optical constants. d) Femtosecond transient absorption spectra according to pump-probe delay time at a pump power of 100  $\mu$ J cm<sup>-2</sup>. e) Maximum material gain coefficients at a pump-probe delay time of 3 ps, which were determined from optical density changes ( $\Delta A + A_0$ ) and film thickness. f) Transient absorption ( $-\Delta A/A_0$ ) decay curve at 630 nm. Dash lines, bi-exponential fitting curve. Dotted line, transparent state at  $-\Delta A/A_0 = 1$ .

The PL quantum yield measured using an integrating sphere was nearly unchanged; 48 ± 2% for ODA-QD and 46 ± 2% for DAO-QD samples. From the decay curves and quantum yield, the radiative transition rate ( $k_r$ ) and nonradiative transition rate ( $k_{rr}$ ) are calculated to be 5.7 × 10<sup>7</sup> and 6.2 × 10<sup>7</sup> s<sup>-1</sup> for ODA-QD and 8.2 × 10<sup>7</sup> and 9.7 × 10<sup>7</sup> s<sup>-1</sup> for DAO-QD, respectively (**Table 1**). Both transition rates were increased ≈1.5-fold after ligand exchange.

Using ellipsometry, the refractive index of ODA-QD films was measured to be 1.76 at a wavelength of 650 nm. The refractive index increased to 1.83 after ligand exchange to DAO-QD (Figure 2c). The moderate 4% increase compared to volume shrinkage may indicate a decreased refractive index of the inter-QD space while the index of the QD core shell ( $n \approx 2.5$ ) is unchanged, but this speculation remains to be verified. The extinction coefficients increased from 0.011 in ODA-QD to 0.015 in DAO-QD. The Q-factor of cavity resonance increases nonlinearly with the index difference with respect to the surrounding.<sup>[4]</sup> Therefore, the increased refractive index is desirable for applications in high-index environments such as tissues and organic solvents. The increased extinction is also desirable as it is related to pump absorption and stimulated emission.

To characterize optical gain, we performed femtosecond pump-probe absorption spectroscopy. Figure 2d shows  $\Delta A + A_0$ spectra as a function of pump-probe delay time at a pump fluence of 100  $\mu$ J cm<sup>-2</sup>, where  $\Delta A$  is transient absorbance and  $A_0$ is ground-state absorbance (Figure S3, Supporting Information). The average  $\Delta A + A_0$  value in a range of 630–635 nm at a pumpprobe delay time of 3 ps was -5.66 mOD for ODA-QD and -5.89 mOD for DAO-QD films. Considering film thicknesses, these values correspond to a gain coefficient of 1510 cm<sup>-1</sup> for ODA-QD and 2150 cm<sup>-1</sup> for DAO-QD (Figure 2e). The 1.4-fold increase of the gain is in a reasonable agreement with the 27% linear size reduction. Pump-probe absorption decay curves typically contain a fast exponential decay component ( $\tau_1$ ) due to stimulated radiative recombination (amplified spontaneous emission) and slower component  $(\tau_2)$  related to Auger recombination. Our pump-probe measurement revealed the fast and slow time constants of 47 and 345 ps for ODA-QD films and 35 and 302 ps for DAO-QD films. The decreased stimulated recombination time is consistent with the increased gain. The decay time to transparency<sup>[29]</sup> or gain lifetime ( $\tau_{\sigma}$ ) was 364 ps for DAO-QD films, 1.5-fold longer than 243 ps for ODA-QD films (Figure 2f). The photophysical properties of QD films are summarized in Table 1.

 Table 1. Photophysical properties of QD thin films.

Material	Quantum yield [%]	$k_r  [s^{-1}]$	$k_{nr}  [s^{-1}]$	<i>n</i> @650 nm	k @614 nm	<i>τ</i> <sub>l</sub> [ps]	$ au_2$ [ps]	$\tau_{\rm g}[{\rm ps}]$	Gain [cm <sup>-1</sup> ]
OAD-QD	48	$5.7 \times 10^7$	$6.2\times10^7$	1.76	0.011	47	345	243	1510
DAO-QD	46	$8.2  imes 10^7$	$9.7  imes 10^7$	1.83	0.015	35	302	364	2150

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**Figure 3.** Structural analysis of QD microbeads. a) SEM of QD microbeads before and after ligand exchange. b) DAO-QD microbeads with a diameter of 1.69 μm. c) 5.54 μm microbeads. d) TEM images of a cross-section of DAO-QD microbead. e) EDS maps of a cross-section of DAO-QD microbead. Scale bars in (a–c), 5 μm. Scale bar in (d–e), 500 nm.

#### 2.3. Structural Analysis of Compact QD Microbeads

Using a microfluidic setup, we produced ODA-QD microbeads with diameters that are tunable by varying flow speeds. **Figure 3**a shows scanning electron microscopy (SEM) images of ODA-QD microbeads fabricated with relatively uniform diameters of  $3.48 \pm 0.02 \ \mu$ m. DAO-QD microbeads maintain spherical shapes with good surface smoothness and reduced diameters of  $2.96 \pm 0.03 \ \mu$ m (Figure 3a). The size reduction is 15% in diameter and of 39% in volume. The same size reduction was measured for microbeads with different diameters (Figure 3b,c; Figure S4 and Table S2, Supporting Information). Highly monodispersed QD microbeads in the same batch showed a lasing wavelength variation of ~1% ( $\Delta\lambda/\lambda$ ) (Figure S5, Supporting Information), which corresponds to the diameter deviation of ~1% ( $\Delta$ d/d) determined by SEM (Table S2, Supporting Information).

50-nm-thick cross-sectional sections of DAO-QD microbeads were prepared using a micromanipulator-equipped focusedion-beam tool. Transmission electron microscopy (TEM) of the samples revealed uniform distribution of core-shell QD throughout the cross-section, indicating uniform ligand exchange in the entire volume (Figure 3d). Elemental mapping by energy dispersive X-ray spectroscopy (EDS) also showed uniformly dispersed QDs throughout the microbeads (Figure 3e).

# 2.4. Stimulated Emission Characteristics of Compact QD Microbeads

The PL spectra of QD microbeads, when excited with continuous-wave pump light at 495 nm, showed cavity modal structures superimposed on dominant, broadband fluorescence (Figure S6, Supporting Information). To investigate stimulated emission characteristics, we used a nanosecond optical parametric oscillator (OPA, 3 ns, 20 Hz) pump laser tuned at 480 nm. **Figure 4**a shows the output emission spectra of two representative ODA-QD and DAO-QD microbeads obtained as the pump fluence was increased. At pump fluences above threshold, single lasing mode emerged (Figure 4b). The dramatic linewidth narrowing and nonlinear kink in light-in-lightout curves clearly indicate lasing (Figure 4c,d). The typical

FWHM linewidth was 0.8 nm before and 0.6 nm after ligand exchange. The threshold fluence was  $1.12 \pm 0.21$  mJ cm<sup>-2</sup> for ODA-QD and 0.85  $\pm$  0.23 mJ cm<sup>-2</sup> for DAO-QD microbeads (Figure 4e). Similar reduction in lasing threshold was obtained for different microbead sizes (Figure S7, Supporting Information). The smallest DAO-QD microbeads that showed lasing in the air were 1.5  $\mu$ m in diameter with a threshold of  $\approx$ 1.3 mJ cm<sup>-2</sup> (Figure 4f,g). These microbeads had a diameter of 1.7 µm prior to ligand exchange, at which lasing could not be reached even with high pump fluences up to 30 mJ cm<sup>-2</sup>. The 1.5  $\mu$ m size represents a fourfold size reduction compared to previous QD-based microlasers reported to date (Table S3, Supporting Information). The resonance wavelengths of the WGM with different polar indices and polarization states were calculated for various microbead diameters.<sup>[4]</sup> Given the mode spacing and lasing bandwidth (≈25 nm), it predicted single-mode lasing of one TE mode for small bead diameters below ~5 µm and multimode lasing for larger (Figure S8, Supporting Information).

**Figure 5**a shows the stability of output emission across prolonged operation at a pump fluence of 1.4 mJ cm<sup>-2</sup> above lasing threshold. While photobleaching is typical for conventional dyedoped polystyrene microspheres, DAO-QD microbeads showed no degradation intensity across 25000 pump pulses, and no measurable spectral shift (Figure S9, Supporting Information). Furthermore, when placed in water and various polar or apolar organic solvents, the crosslinked microbeads with diameters of ~5.5 µm stably maintained single laser peaks (Figure 5b). The lasing wavelength is different for different media because of their refractive indices and deviation of bead diameter. QD microbeads maintained single-mode emission when embedded in solid polymer resins, such as polydimethylsiloxane (PDMS, n = 1.42), epoxy (n = 1.51), and polystyrene (n = 1.59) (Figure 5c).

#### 2.5. Spectral Multiplexing of Single-Mode Microbead Lasers

To assess color multiplexing, we embedded DAO-QD microbeads with slightly varying diameters in a gel (Figure 6a). Spectral images of the microbeads were acquired with pump fluences below and above threshold, respectively. Each image pixel (1  $\mu$ m × 1  $\mu$ m) that has PL signals above detection noise was color-coded according to center wavelength determined





**Figure 4.** Stimulated emission characteristics of microbeads. a) Contour plots of PL spectra of a single ODA-QD microbead (left) and a DAO-QD microbead (right). b) PL spectra of microbeads at pump fluences below (0.5 mJ cm<sup>-2</sup>; black, 10× magnified in intensity) and above (2 mJ cm<sup>-2</sup>; magenta) lasing threshold. c) Spectral linewidths of two microbeads. d) Output intensity curves of microbeads. e) Lasing threshold fluences (N = 10 each). f) SEM image of the smallest lasing DAO-QD microbead with a diameter of 1.5 µm. g) Output intensity curve of the 1.5 µm microbead. Inset, single-mode lasing spectrum.

by Gaussian curve-fitting of the detected spectrum at the location. All the microbeads had the same PL center wavelength at  $636 \pm 0.2$  nm below lasing threshold. However, above threshold the lasing peaks of microbeads are more diverse across a range from 640 to 658 nm (Figure 6b). A total of 19 different colors with an interval of 1 nm are generated (Figure 6c).

The data suggest a remarkable advantage of the microbeads for spectral multiplexing. Considering the wide tunability of II-VI QD gain materials over 400 to 1600 nm, more than 1000 colors (1 nm step) can in principle be generated even without combinatorial or intensity encoding.<sup>[30]</sup> The accuracy of center wavelength measurement is approximately proportional to the square of the ratio of the spectral bin size to the linewidth. This color scheme is robust because the spectral bin size of 1 nm is larger than the 0.6 nm linewidth. However, for broadband fluorescence with a FWHM of 30 nm, the narrowband color scheme does not work reliably because it would require a 2500-fold higher signal to noise ratio (SNR) and, moreover, it is vulnerable to any external factors that can distort the detected spectra, such as wavelength-dependent absorption and scattering in the surroundings.

#### 2.6. Imaging Single-Mode Microbead Lasers In Vitro and In Vivo

To test potential as imaging probes, we performed in vitro and in vivo imaging experiments. Murine breast cancer 4T1 cells were incubated with DAO-QD microbeads with a diameter of  $\approx$ 4 µm to induce intracellular uptake of the microbeads (**Figure 7**a). A cell viability assay using cell-counting kit-8 (CCK-8) confirmed negligible cytotoxicity of microbeads (Figure S10, Supporting Information).<sup>[31]</sup> The microbead-tagged cells were traced over 3 h (Figure 7b and Figure S11, Supporting Information), during which the intracellular microbeads was stable with wavelength variations less than 0.3 nm (Figure 7c). Wavelength changes arise from sensitivity to the surrounding refractive index changes by internal cellular process or random movement of the microbead in the cytoplasm.

Next, we injected microbeads subcutaneously in the abdomen of an anesthetized, live mouse (BALB/c strain). Twophoton microscopy using 140-fs pulses at 920 nm visualized the microbeads via both two-photon excited fluorescence and second harmonic generation (SHG) at a depth of  $\approx$ 90 µm from the skin surface (Figure 7d). We then imaged the sample using



**Figure 5.** Stable single-mode lasing in various media. a) Laser peak intensity of a DAO-QD microbead and dye-doped polystyrene bead across 25000 pump pulses (1.4 mJ cm<sup>-2</sup>, 20 Hz repetition for 21 min). b) Lasing spectra of DAO-QD microbeads with diameters of  $\approx$ 5.5 µm immersed in different media. c) Photo of a PDMS slab containing microbeads under UV illumination.

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Figure 6. Multicolor imaging of microbead lasers in Matrigel. a) Bright-field image. b) Laser emission microscopy images with pump fluences below and above threshold. Color represents center wavelength. Scale bars, 50 µm. c) Center wavelength distribution of 140 microbeads below and above threshold.

480-nm nanosecond pump (Figure S12, Supporting Information). The single-peak stimulated emission from individual microbeads was measured with high SNR (Figure 7e). By comparison, dye-doped polystyrene microbeads produced broadband fluorescence but failed to reach threshold of lasing inside tissues despite their larger size of 10  $\mu$ m (Figure S13, Supporting Information).

## 3. Conclusion

The novel microbeads made of densely packed, crosslinked QDs are well suited for stimulated emission. Although bulk semiconductor crystals in the III-V group have high refractive indices (n > 3.0), the processes to fabricate them into microparticles are relatively laborious involving epitaxial growth on wafers, ion etching, wet etching, and clad coating,<sup>[10]</sup> and it is quite difficult to make them in spherical geometry. II-VI

semiconductor QDs offer higher optical gain than III-V bulk semiconductors as individual nanocrystals, and results in high gain (>2000 cm<sup>-1</sup>) in microbeads including the volume occupied by crosslinking ligands. The refractive index of II-VI material is typically 2.4 to 2.7 in visible wavelengths.<sup>[32]</sup> The average refractive index of microbeads is lower (n = 1.83) due to the presence of ligands. Nonetheless, semiconductor QDs and in situ crosslinking via ligand exchange are attractive building blocks for fabricating compact lasing microbeads, as we have demonstrated in this work.

The most remarkable feature of the QD microbeads is its single-peak stimulated emission with sub-nanometer linewidth. This gives much enhanced spectral contrast and sharpness compared to fluorescence or spontaneous emission. While previously reported spherical microlasers have almost exclusively generated multiple spectral peaks, the compact QD microbeads demonstrated here predominantly generate a single laser peak, tunable by diameter across a gain bandwidth of ≈19 nm.



**Figure 7.** In vitro and in vivo imaging of lasing microbeads. a) Fluorescence images of DAO-QD microbeads (red) in 4T1 cells. Scale bar, 100 μm. b) Bright-field images and time lapse trajectories of three microbeads, labeled 1 to 3, in 4T1 cells. c) Lasing wavelengths of the three intracellular microbeads. d) Two-photon excitation fluorescence images of subcutaneously injected microbeads in a mouse in vivo, showing three microbeads, labeled 4 to 6. e) Emission spectra of the microbeads in comparison to a polystyrene microsphere with a size of 10 μm in tissue. Scale bars: 20 μm in (b,d).



Single-mode laser emission was obtained with microbead diameters in a range from 1.5 to 5 µm. For sizes smaller than 1.5 µm, lasing threshold could not be reached. Larger diameters than 5 µm tend to produce two or multiple spectral peaks. The single-mode microbeads provided 19 color channels with a spacing of 1 nm, which is greater than the individual emission linewidth of 0.6 nm. Using QDs with different core and shell sizes and alloy compositions, it is in principle possible to generate ≈1000 (600 nm/0.6 nm) color channels across the visible and near-IR wavelengths. Currently, the lasing wavelength is sensitive to the refractive index of the surrounding medium because the optical field of the cavity mode extends into the medium. This sensitivity can be greatly reduced by surface coating around the microbeads with low-index material.<sup>[10]</sup> On the other hand, different surface designs may be incorporated to increase the sensitivity to the environment and certain biomolecules deliberately for biosensing.<sup>[4,20,33]</sup> The amine termination of the organic ligand would render facile chemical functionalization of the microbead surface.

The single-mode lasing microbeads have potential to replace conventional fluorescent microbeads in many applications including multiplex assays, imaging probes, drug screening, and combinatorial chemistry, with  $\approx$ 100-fold enhanced spectral density and color-multiplexing capability.

### 4. Experimental Section

Materials: CdSe/ZnS core shell QDs (QSP-620) were purchased from Ocean Nanotech. DAO, 1,8-octanedioic acid, 1,8-octanediphosphonic acid, 1,8-octanedithiol, 1,2-ethylenediamine, poly(vinyl alcohol) (PVA,  $M_w$  13000–23000, 87–89%) were purchased from Sigma-Aldrich. 10  $\mu$ m Nile red doped polystyrene beads were purchased from Microspheres-Nanospheres.

Microsphere Fabrication and Ligand Exchange: The microdroplet generation was done with glass microfluidic chips (14 µm channel size, Dolomite) connected to pressure pumps (Flow EZ, Fluigent), using 5 wt% QDs in toluene as oil phase and 2 wt% PVA in water as continuous phase, forming an oil-in-water emulsion. The emulsion was agitated for 12 h with 500 rpm speed in a mixer until the toluene in oil phase fully evaporated. Solidified QD microspheres were washed by three rounds of centrifugation (1000 rcf, 2 min) and resuspended in water to remove residual surfactants. Microsphere size was controlled by adjusting the flow rates of the oil phase and continuous phase. For ligand exchange, ODA-QD microbeads were centrifuged down (1000 rcf, 2 min), supernatant was removed, and then 1 wt% ligand in 1 mL methanol was introduced, followed by 500 rpm vortexing for 20 min. After 20 min, DAO-QD microbeads were washed by centrifugation (1000 rcf, 2 min) and resuspension in methanol and water to remove residual ligand. All processes were performed in glass vials.

*Optical Characterizations of Thin Films*: A frequency-doubled 382 nm picosecond pulsed laser (VisIR-765, PicoQuant), a photon counting avalanche photodiode (PDM series, Micro Photonics Devices), and spectrograph (shamrock 303i, Andor) were used for transient PL measurements. A variable-angle spectroscopic ellipsometer (J.A. Woollam M-2000FI) was used to measure the optical constants and thickness of QD thin films. The incident angle was varied from 55° to 75° in steps of 5°. The collected data were analyzed using Complete EASE software (J.A. Woollam Co. Inc.). A stylus profilometer was used to measure the thickness of the films. Femtosecond pump-probe absorption spectroscopt was performed with a femtosecond pulsed laser and transient absorption spectrometer (Helios, Ultrafast Systems). Ti:Sapphire regenerative amplifier (Spitfire, Spectra-Physics) and optical parametric amplifier (TOPAS, Spectra-Physics) were used as a laser light

source. The pump beam was 400 nm in wavelength and had a fluence of 100  $\mu$ J cm<sup>-2</sup>. Repetition rates of the probe and pump beam were 1000 and 500 Hz, respectively.

*Electron Microscopy*: SEM images were obtained using a Phenom Pharos (Nanoscience) at 5 kV. Cross-sectional TEM samples were prepared using an in situ lift-out technique with a focused-ion-beam tool (Helios Nanolab, FEI Company) with final Ga<sup>+</sup> milling performed at 5 keV. TEM images and EDS maps were obtained using FEI Talos 200× TEM/STEM.

Optical Characterizations of Microbeads: A custom-built hyperspectral microscope was used for PL measurements (Figure S14, Supporting Information). The setup used a 50×, 0.6-NA air objective lens (Nikon), a motorized XY translational sample stage (MLS-203, Thorlabs), a nanosecond pump laser (Opolette HE 355 LD, Optotek), an EMCCD camera for wide-field imaging, and an EMCCD-coupled spectrometer (Newton, Andor) with a spectral resolution of 0.1 nm. For multi-color imaging, microbeads were embedded in Matrigels in a 96-well plate. Hyperspectral images were a 300  $\mu$ m by 300  $\mu$ m field of view by using the custom-built hyperspectral microscope. The emission spectra were measured at each image pixel (1  $\mu$ m  $\times$  1  $\mu$ m) that has PL signals above detection noise was color-coded according to center wavelength determined by Gaussian curve-fitting of the detected spectrum at the location.

Cell Culture In Vitro Experiments: Murine mammary carcinoma 4T1 cell-line cells, Hela human cervical cancer cell-line cells, and L929 murine fibroblast cell-line cells (from ATCC) were cultured in Dulbecco's modified Eagle medium media supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin-streptomycin at 37 °C in a humidified CO<sub>2</sub> incubator. For cytotoxicity evaluation, Hela cells and L929 cells were seeded in a 96-well plate at ≈10000 cells per well and incubated for 24 h in advance. Then, cells were immersed with fresh whole media containing different numbers of microbeads and incubated for another 24, 48, and 72 h. Cell viability was guantified using CCK-8 assay (ApexBio), with untreated cells as a control, normalization group and background from wells with medium only. For fluorescence imaging, cells after fixation (4% paraformaldehyde solution) were stained with Cell Tracker Green CMFDA (Thermo Scientific) for cytoplasmic labeling and 4',6-diamidino-2-phenylindole (Thermo Scientific) for nuclear labeling, according to manufacturer's guidelines.

Imaging Microbeads in Mice: The MGH Institutional Animal Care and Use Committee approved animal protocol (2017N000021) in accordance with NIH guidelines. BALB/c mice aged 8-10 weeks were purchased from Jackson Laboratories. A mouse was initially anesthetized using isoflurane gas. Its body temperature was maintained at 37 °C, and ophthalmic ointment was applied to both eyes to protect eye drying. The hair in the abdominal skin was removed using hair removal cream. Microbeads in 30 ul Dulbecco's phosphate-buffered saline solution were subcutaneously injected into the abdomen of the mouse. After 6 h of injection, the mouse was anesthetized again using a ketamine-xylazine mixture (100 mg/10 mg per kg bodyweight) and transferred to microscopes for imaging. A laser-scanning intravital two-photon microscope IVM-MS (IVIM Technology, Daejeon, South Korea) equipped with a femtosecond pulsed fiber laser (920 nm, <140 fs, 80 MHz) was used to image QDs and fluorescent microbeads. Band pass filters for 461/5 and 647/57 nm were used to detect SHG and far-red signal, respectively. The custom hyperspectral microscope equipped with the nanosecond pump laser was used to characterize laser emission.

## **Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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### **Conflict of Interest**

S.-H.Y. has financial interests in LASE Innovation Inc., a company focused on commercializing technologies based on laser particles that were reviewed and are managed by Massachusetts General Hospital and Partners HealthCare in accordance with their conflict of interest policies. All other authors declare no conflict of interest.

### **Data Availability Statement**

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

### **Keywords**

bio-imaging, colloidal quantum dots, ligand exchange, microbeads, microlasers, multiplexing, stimulated emission

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