

Compact and Filter-Free Luminescence Biosensor for Mobile *in Vitro* Diagnoses

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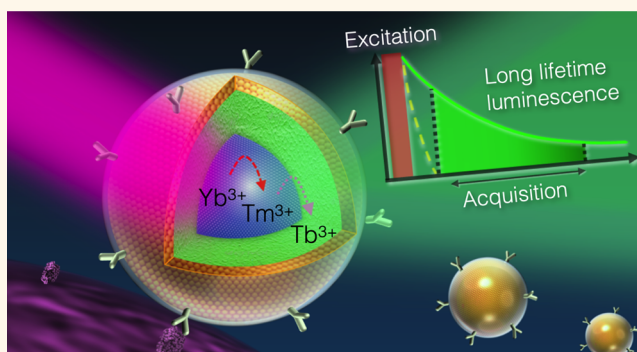
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Supporting Information

ABSTRACT: We report a sensitive and versatile biosensing approach, LUCID (luminescence compact *in vitro* diagnostics), for quantitative molecular and cellular analyses. LUCID uses upconversion nanoparticles (UCNPs) as luminescent reporters in mutually exclusive photoexcitation and read-out sequences implemented on a smartphone. The strategy improves imaging signal-to-noise ratios, eliminating interference from excitation sources and minimizing autofluorescence, and thus enables filterless imaging. Here we developed a miniaturized detection system and optimized UCNPs for the system and biological applications. Nanoparticle luminescence lifetime was extended by controlling particle structure and composition.

When tested with a range of biological targets, LUCID achieved high detection sensitivity (0.5 pM for protein and 0.1 pM for nucleic acids), differentiated bacterial samples, and allowed profiling of cells. In proof-of-concept clinical use, LUCID demonstrated effective screening of cancer cells in cervical brushing specimens, identifying patients at high risk for malignancy. These results suggest that LUCID could serve as a broadly applicable and inexpensive diagnostic platform.

KEYWORDS: diagnostic, point-of-care, nanoparticles, luminescence, global health



Infections and cancer are leading causes of death in low and middle income countries (LMICs), with annual death tolls reaching over 7 million (infection, 4.7 million; cancer, 2.4 million).¹ Access to treatment of preventable and curable diseases has significantly improved in many LMIC settings, spurred by continuing global initiatives.^{2,3} Reaping the full benefit of these advances mandates access to affordable diagnostic tests to enable prompt initiation of treatment or expedited patient triaging. Limited infrastructure and geographical barriers, however, demand stringent performance metrics on LMIC diagnostics.^{4,5} Assays should yield actionable results preferably at the point-of-care (POC). Moreover, sensing systems should be easy to operate and maintain and also be compact for use in mobile clinics. We further reason that ideal POC sensors should be platform technologies equipped to deal with diverse targets in the field settings. Different sensor types have been advanced for POC

operations, yet typically limited to specific analytic targets. For example, low-cost membrane-based sensors allow for low-resolution, qualitative measurement of soluble molecules (e.g., serum proteins, nucleic acids).^{6–8} For cellular detection, miniaturized imaging devices have been developed,^{9–12} although their operation often relies on intensive computation,¹³ complex optics (e.g., fluorescence, illumination control),^{11,14} or external servers.¹⁵

We set out to devise an end-user-friendly, generalizable strategy for quantitative molecular tests. We specifically explored upconversion nanoparticles (UCNPs) in an imaging-based assay, both for soluble targets, bacterial and cellular detection. UCNPs have a distinct optical feature—they emit

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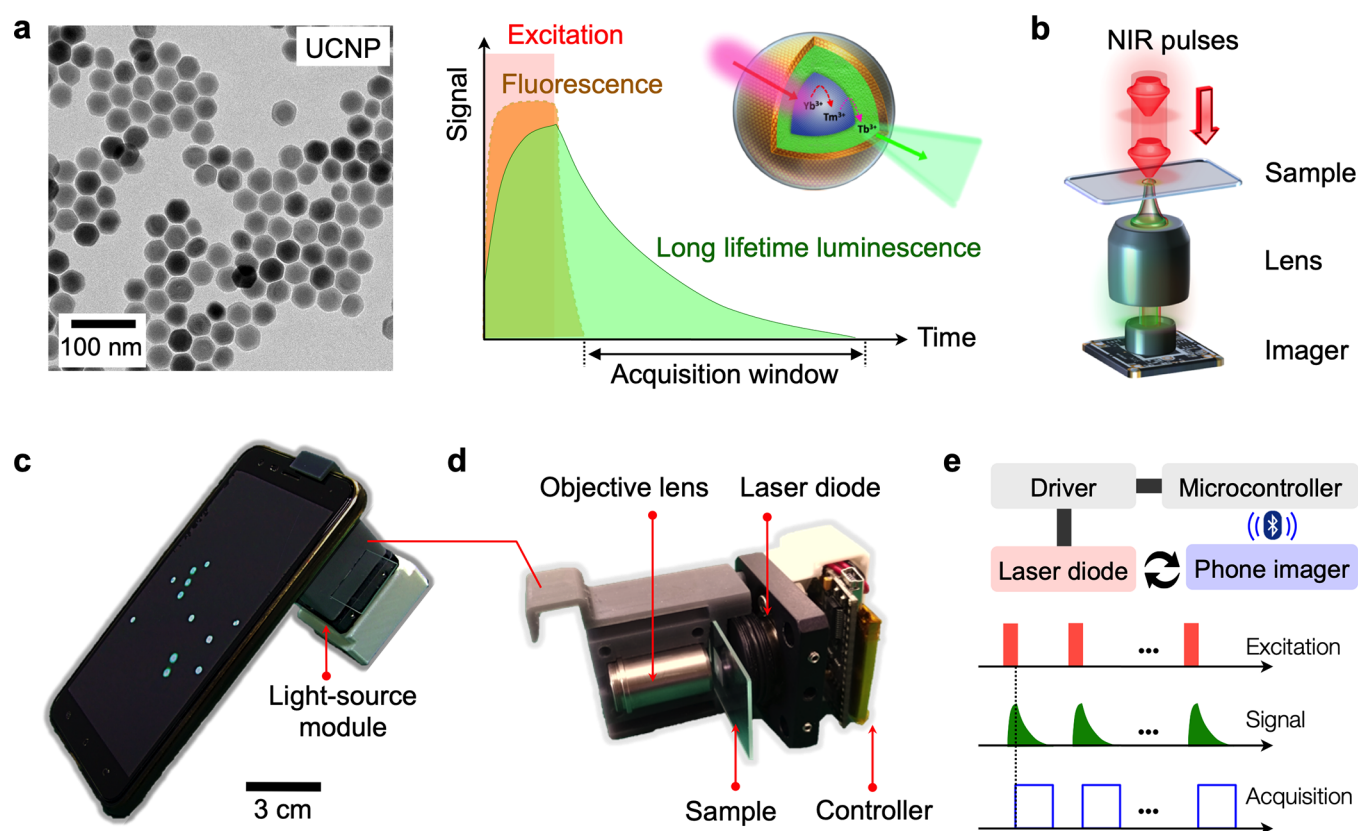


Figure 1. Luminescence upconversion *in vitro* diagnostics (LUCID). (a) The assay used upconversion nanoparticles (UCNPs; left) as reporters. Optical excitation and detection were temporally separated, as the particles can emit luminescent light after pulse excitation (right). (b) The LUCID scheme simplified the detection optical setup. An imager can directly image UCNPs-labeled samples without using filter sets. (c) A mobile LUCID prototype was assembled by attaching an excitation module to a smartphone camera. (d) The excitation module consisted of a laser diode (emission, 980 nm), its control electronics, and beam shaping optics. (e) A microcontroller provided the triggering cues for particle excitation and image acquisition, ensuring that only the luminescence signal was recorded. Multiple frames could be averaged to improve overall image quality. For the prototype shown in (c), the controller communicated with the phone via Bluetooth to access its camera function.

luminescent light even after photoexcitation is turned off,^{16–18} allowing for temporal decoupling between optical excitation and detection. Such imaging would be free from optical crosstalk and detector-saturation caused by high-intensity illumination. This in turn simplifies a system setup by enabling filterless imaging. Based on this principle, here we report on a compact luminescence platform, termed LUCID (luminescence compact *in vitro* diagnostics), for versatile POC molecular testing. We optimized both nanoparticles and their detection system. Specifically, we lengthened UCNPs' luminescence lifetime by controlling particle composition and built a portable, filterless imaging device around a smartphone. Applying the first prototype, we demonstrate highly sensitive detection across broad ranges of biological targets. LUCID detected soluble proteins (~ 0.5 pM) and nucleic acids (~ 0.1 pM) at high sensitivity, identified different bacterial species, and screened individual cancer cells. As for potential clinical applications, we used LUCID in the context of cervical cancer screening. Specimens obtained during colposcopy were subjected to LUCID for cellular detection, which reliably identified patients at high clinical risk for malignancy.

RESULTS AND DISCUSSION

LUCID Strategy and System Development. Photo-excited UCNPs emit both fluorescent and luminescent lights; fluorescence decays (<100 ns) soon after excitation is

removed, whereas luminescence typically persists (Figure 1a). LUCID is designed to detect this long-lived luminescence only, which brings the following advantages: (i) imaging becomes free of artifacts associated with fluorescence detection (e.g., no bleed-through of excitation light, autofluorescence, photobleaching); (ii) repeated imaging can selectively amplify luminescence signal without increasing the background; and (iii) samples are directly imaged without need for optical filter sets (Figure 1b).

These merits enabled us to devise a compact, mobile LUCID system (Figures 1c and Supplementary Figure 1). Figure 1c shows a prototype built with a smartphone. We used a phone camera as a detector and interfaced it with an illumination source (Figure 1d). A snap-on module, housing a laser diode, optical lenses, and a microcontroller, was assembled and mounted over the phone camera. The microcontroller was programmed to synchronize operations between the laser diode and the phone camera, ensuring image acquisitions when the diode was turned off (Figure 1e). Specifically, the detection cycle started with NIR illumination (~ 5 ms). Excitation light was then turned off; the detector acquired the luminescence signal after a short time-delay (1 ms). The integration time was set to $\sim 3 \times$ the luminescence lifetime (τ), capturing $>90\%$ of the luminescence light.

Development of Nanoreagents. We next optimized UCNPs for LUCID applications, focusing on extending

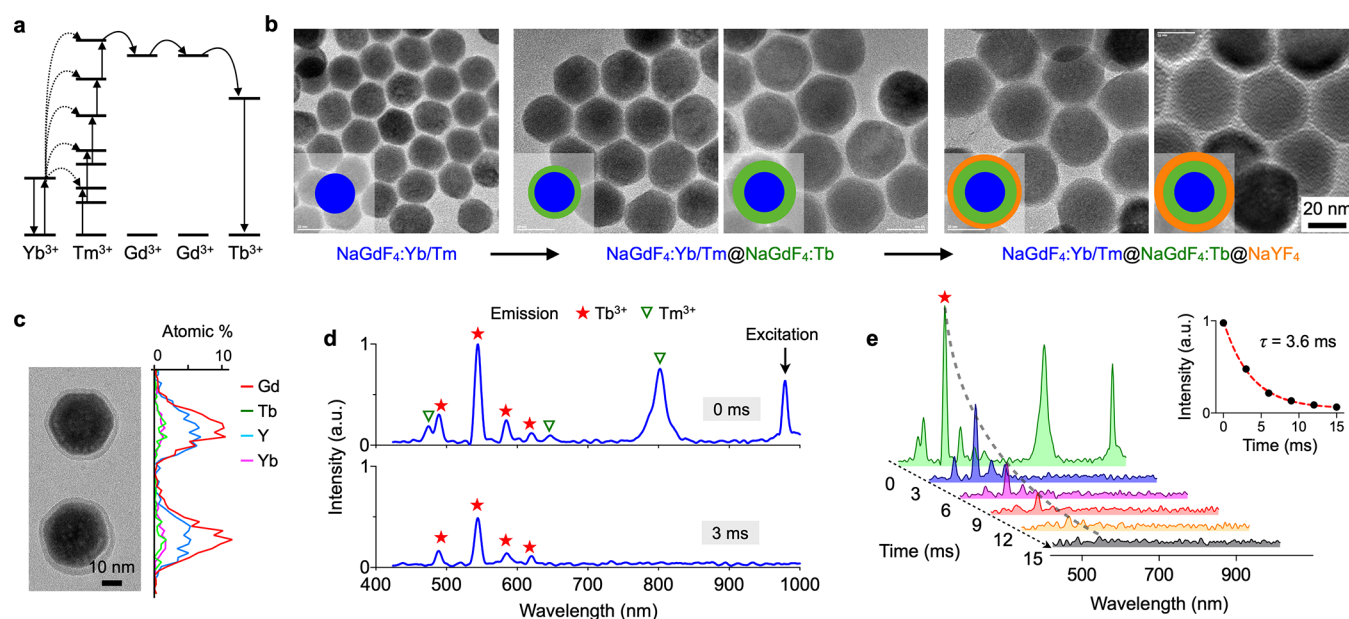


Figure 2. UCNP optimized for LUCID. (a) Energy diagram. Yb^{3+} functions as a sensitizer to absorb NIR photons. The energy is accumulated in Tm^{3+} and then transferred to Tb^{3+} through Gd^{3+} . The particle finally emits photons with Tb^{3+} as an activator. (b) Synthetic route. We first synthesized a core particle ($\text{NaGdF}_4\text{:Yb/Tm}$) and then grew a shell of emission layer ($\text{NaGdF}_4\text{:Tb}$). The particle was further passivated with a protection layer (NaYF_4). (c) Energy dispersive X-ray spectroscopy (EDS) line scan confirmed the incorporation of key elements (i.e., Gd, Tb, Y, Yb). (d) The emission spectra of the prepared UCNP were measured. With the excitation light on, the particle showed emission peaks both from Tm^{3+} and Tb^{3+} (top). With the excitation off, the short-lived Tm^{3+} luminescence disappeared, but the signal from Tb^{3+} persisted (bottom). (e) Time-lapse detection revealed persistent luminescence from Tb^{3+} . The intensity was fitted to a single-phase exponential decay function (inset). The decay time was 3.6 ± 0.4 ms.

luminescence lifetime. Conventional UCNP tend to have short luminescence lifetimes ($\sim$ microseconds).^{19,20} Further extending luminescence lifetime is desirable; this can relax requirements for high-speed electronics and reduce electronic noise through longer signal integration. With a typical semiconductor imager, we estimated that the required luminescence lifetime should be in the range of milliseconds (see [Supporting Information](#) for details). As a luminescence source, we selected Tb^{3+} which is known to have a long luminescence lifetime (several milliseconds).^{16,21} The material, however, required deep UV (254 nm) excitation, which would increase device costs (i.e., UV light source) and raise safety concerns. To provide UV-level excitation from NIR, we embedded Tb^{3+} into a UCNP substrate. Specifically, we used a $\text{Yb}^{3+}/\text{Tm}^{3+}$ codoped NaGdF_4 as a core material; this particle can absorb 980 nm NIR light and emit deep UV photons through the upconversion process ([Figure 2a](#)). A Tb^{3+} doped shell was then grown around the core as an emitting layer.

[Figure 2b](#) shows the synthetic route. We first formed $\text{NaGdF}_4\text{:Yb/Tm}$ core particles (diameter, 20 nm) via a thermal decomposition method (see [Experimental Section](#)).^{22,23} Next, we encased the core with a shell of $\text{NaGdF}_4\text{:Tb}$. Through a dual-growth step, we thickened the shell (thickness, 5.8 nm) to enhance overall emission intensity. We finally passivated the particle with an inert NaYF_4 shell to protect Tb^{3+} from vibrational quenching by solvents. The particle had the overall diameter of 37 nm and showed high crystallinity ([Supplementary Figure 2a](#)) from epitaxial growth. Elemental mapping confirmed the incorporation of Tb^{3+} into the UCNP matrix ([Figure 2c](#) and [Supplementary Figure 2](#)). Synthesized UCNP were made water-soluble through ligand exchange.^{24,25} We incubated oleic-acid-capped UCNP with excess amounts of

poly(acrylic acid) (PAA). The carboxylic group on PAA was used for bioconjugation ([Experimental Section](#)).

We analyzed the optical properties of the prepared UCNP. Aqueous suspensions of UCNP were pulse-excited at 980 nm, and resulting emission spectra were measured. During excitation, we observed emission peaks from both Tm^{3+} (core) and Tb^{3+} (shell). The core emission (Tm^{3+}) had two major emission peaks from internal transitions (475 and 800 nm). These peaks decayed fast (<1 ms); the reported lifetimes were ~ 100 μs (475 nm) and ~ 340 μs (800 nm).²⁶ In contrast, Tb^{3+} emission peaks persisted much longer after excitation was turned off ([Figure 2d](#)). We determined Tb^{3+} luminescence lifetime via time-lapse measurements ([Figure 2e](#)). The emission at 546 nm, which is characteristic of Tb^{3+} , showed an exponential decay ([Figure 2e](#), inset) with the lifetime $\tau = 3.6 \pm 0.4$ ms. This value was about 8-fold higher than the luminescence lifetime of conventional UCNP ($\text{NaYF}_4\text{:Yb/Tm}$; [Supplementary Figure 3](#)).

Platform Characterization. We first tested LUCID using UCNP-coated microbeads (diameter, 10 μm). We compared bead images obtained through conventional fluorescence detection and LUCID ([Figure 3a](#)). Fluorescence images had lower signal-to-noise ratio ($\text{SNR} = 2.6$), likely due to the bleed-through from incident light. In contrast, LUCID was nearly background free, achieving higher SNR ($= 7.3$) even from single image acquisition (15 ms integration time). We further improved LUCID SNR through repeated time-gated imaging. For a given repetition number of N , the signal level would be linearly proportional to N , whereas system noise (mainly from dark currents of an imager) increases as $N^{1/2}$. Overall SNR is thus expected to scale as $N^{1/2}$. Repeated measurements on UCNP-coated microbeads confirmed such reasoning ([Figure 3b](#)) with overall SNR increasing ($\sim N^{1/2}$) with repeated

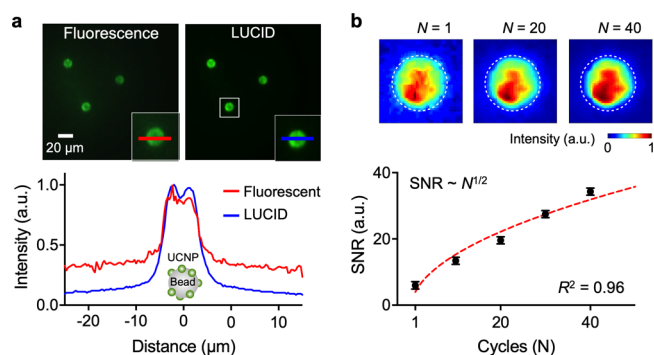


Figure 3. Characterization of LUCID signal. (a) Polystyrene microbeads (diameter 10 μm) were embedded with UCNPs and imaged in conventional fluorescence and LUCID detection modes (top). LUCID had lower background than fluorescence imaging, free from autofluorescence and scattering of incident light. The graph (bottom) shows intensity line scans of the same bead under the two detection modes. The signal level was normalized to its maximum. (b) The overall signal-to-noise ratio (SNR) of LUCID improved by repeating the luminescence imaging sequence (Figure 1e). The SNR scaled as $N^{1/2}$, where N is the cycle number. Each data point is from five beads, and data is displayed as mean $\pm$ SD. The inset (top) shows pseudocolored images of an UCNP-embedded microbead (dotted circle).

excitation and integration. Note that this type of measurement was possible due to the high photostability of UCNPs (i.e., no photobleaching).

LUCID Molecular Assay Formats. We next adopted LUCID to detect different types of biological targets. We developed assays for three different types of targets: (i) soluble protein, (ii) bacterial RNA, and (iii) whole cells. For small molecular targets (e.g., soluble protein, nucleic acid), we used microbeads (diameter, 10 μm) as a solid substrate to capture molecules and subsequently label them with UCNPs (Supplementary Figure 4a). This approach effectively enhances detectable luminescence signal by locally concentrating UCNPs. The assay also benefits from three-dimensional diffusion of beads and target analytes, which shortens the traveling distance of targets before they are captured by affinity ligands. For cellular detection, we used cells as a substrate and directly labeled them with antibody-coated UCNPs.

We acquired multiple time-gated frames ($N = 20$) to produce a final image. For each frame, the laser diode was on for 5 ms; after 1 ms delay, signal was acquired for 10 ms. The total light illumination was short (~ 0.1 s) with no apparent thermal effect on samples. After the image acquisition, computing routines automatically located UCNP-labeled objects, either beads or cells, according to their light intensity, and calculated total intensity per object. As an analytical measure, we used the mean intensity value from ≥ 25 beads or 50 cells (see Experimental Section). A low magnification lens ($20\times$) was sufficient for imaging while extending the system's field-of-view ($350 \times 350 \mu\text{m}^2$).

For small molecule detection, we used thrombin and bacterial DNA as model targets. We conjugated microbeads with capture ligands: aptamers for thrombin (Supplementary Table 1) and oligonucleotides complementary to bacterial DNA. UCNPs were also modified with target-specific affinity ligands (see Experimental Section). To assess assay statistics, we generated calibration curves (signal vs analyte concentration) using standard samples of known analyte concen-

trations (Supplementary Figure 4b and c). Negative controls were prepared by incubating beads with UCNPs but without targets. LUCID achieved high sensitivity with a limit-of-detection (LOD) of 0.5 pM for thrombin and 0.1 pM for DNA. The dynamic range spanned about 3 orders of magnitude.

We extended the assay to pathogen analyses. A set of oligonucleotide pairs were prepared to detect different bacterial species. The oligonucleotides were complementary to the hypervariable region of 16S rRNA across different bacterial species (Supplementary Table 2).²⁷ RNA was extracted from bacteria samples and PCR-amplified to produce single-stranded DNA. Figure 4a shows a representative

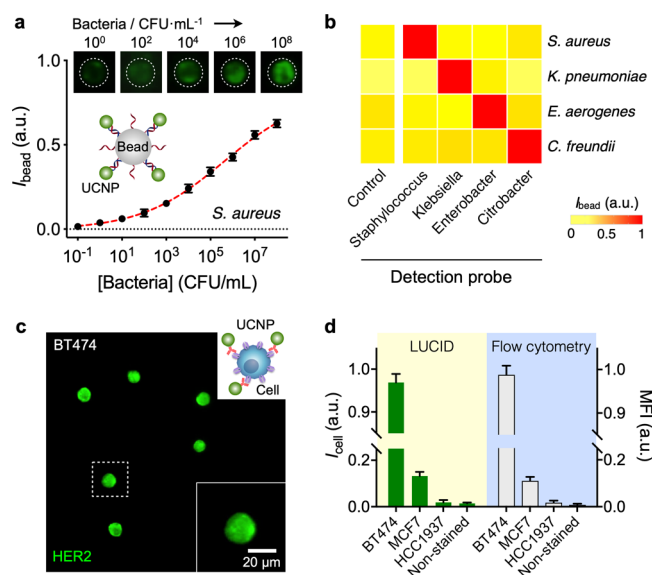


Figure 4. Versatile LUCID assays. (a) Bacteria detection. DNA targets from *S. aureus* were detected by LUCID. The detection limit reached down to single colony forming units (CFUs). Data are displayed as mean $\pm$ SD from triplicate measurements. The signal level was normalized to the maximum pixel intensity of the camera. (b) We further designed probe sets for four different bacteria genera (*Staphylococcus*, *Klebsiella*, *Enterobacter*, *Citrobacter*). LUCID displayed excellent signal contrast and achieved high specificity. For each sample, intensities from 25 beads were averaged. (c) Cell profiling. A panel of breast cancer cells (BT474, MCF7, HCC1937) were labeled with UCNPs conjugated with anti-HER2 antibodies, and imaged by LUCID. An image of BT474 cells is shown here; images of other cell lines are in Supplementary Figure 6. (d) LUCID results enabled quantitative cell profiling. The intensity (I_{cell}) of UCNP-labeled cells was reflective of HER2 expression and correlated ($R^2 = 0.99$) with the mean fluorescent intensity (MFI) from flow cytometry. Intensities from 50 (LUCID) and 10^4 (flow cytometry) cells were averaged, and the data are displayed as mean $\pm$ SD.

example, the detection of *Staphylococcus aureus*. Titration experiments showed that the sensitivity was close to single colony forming units (CFUs). We next applied the assay to identify different bacterial species (Figure 4b). LUCID showed high detection selectivity, superior to that of conventional SBYR green-based PCR (Supplementary Figure 5). This could be attributed to the sandwich hybridization employed in the UCNP assay; both capture and detection probes should bind to their target to emit the luminescence signal.

We finally demonstrated cell profiling with LUCID. Human breast cancer cell lines (BT474, MCF7, HCC1937) were labeled with immuno-UCNPs recognizing the human epidermal growth factor receptor 2 (HER2)/*neu*. Differential luminescence signal was observed per cell line (Figure 4c and Supplementary Figure 6), reflective of varying HER2 levels. The average luminescence signal (from 50 cells) correlated linearly with mean fluorescence intensity (MFI) by flow cytometry ($R^2 = 0.99$; Figure 4d).

LUCID Application with Clinical Specimens. To explore LUCID's potential for clinical application, we identified effective yet feasible cervical cancer screening as an unmet need in point-of-care settings. Cervical cancer incurs high incidence and mortality rates in many resource-limited countries²⁸ which often lack robust early screening infrastructures. We reasoned that the portable LUCID system could effectively triage suspicious or high-risk cases. This decentralized strategy could potentially offset pathology bottlenecks and reduce repeated patient visits to central clinics, often complicated by geographical and/or socioeconomic constraint.²⁹

We obtained excess cervical specimens through brushings or biopsies during colposcopic evaluation. Eleven patients with previously abnormal Pap smear results were recruited. We labeled cellular samples with a cocktail of UCNPs specific to EpCAM, CD44 or Trop2; these markers were chosen for their elevated expression in cervical cancer as described in the literature and Human Protein Atlas.^{30–32} LUCID resolved individual cells (Figure 5a) and was able to quantify their luminescence intensities. Patient specimens were also independently assessed via conventional cytopathology,

which classified them into three groups: “high risk”, “low risk”, or “benign” (Figure 5b, top row). On average, we observed higher luminescence signal with increased clinical risk (Figure 5b). Notably, the mean intensity per cell (I_{cell}) could serve as a classification metric (Figure 5c), significantly different among risk groups (Sidak's multiple comparison test).

CONCLUSIONS

Deploying low-cost mobile diagnostics can mitigate healthcare disparities in LMIC settings where pathology bottlenecks, limited resources, and logistic barriers delay disease diagnoses and often lead to over/undertreatment.³³ Such tests would also benefit underserved populations in developed countries; in the United States, for example, a quarter of the population live in rural areas, but only 10% of physicians practice there.³⁴ The developed LUCID platform can help tackle diagnostic challenges in such regions: (i) sensing is robust against autofluorescence and optical scattering; (ii) filter-free, simple optics renders the assay system cost-effective and easy to maintain, and (iii) the platform can perform onsite detection of diverse molecular targets. The underlying technical innovation sought to engineer nanoparticles with long-lived luminescence and integrate them into compact, time-gated imaging devices. Our pilot studies confirmed that LUCID's analytical results are comparable or superior to costlier laboratory tests demanding greater skill sets (i.e., qPCR, flow cytometry, cytopathology).

We used upconversion nanoparticles (UCNPs) that have emerged as a new class of nanomaterials for biosensing.^{35,36} Doped with lanthanide ions, UCNPs absorb multiple near-infrared (NIR) photons and emit visible or ultraviolet (UV) light. The parity forbidden nature of 4f–4f transitions of lanthanide ions delays the spontaneous photon emission, opening a temporal window to excitation-free imaging. Luminescence is also highly stable without photobleaching or blinking.^{37,38} These favorable optical properties have motivated diverse applications of UCNPs for *in vivo* imaging.^{17,20,39–42} One drawback of UCNPs is their low quantum yields (QYs; generally <1%).⁴³ We expect that our Tb-doped UCNPs have a similar QY, although the exact value is yet to be determined. UCNP QYs increase with higher excitation power⁴⁴ but at the risk of photothermal damage on samples. In LUCID, we compensated for the UCNPs' low QY through repeated time-gated imaging (Figure 1e), exploiting the particles' high photostability. Indeed, signal quality progressively improved with acquisition numbers (Figure 3b). Furthermore, by syncing the light source to the camera, the total acquisition time was <2 s.

The current system can be further refined, particularly through particle engineering. First, we envision synthesizing a panel of UCNPs for multiplexed molecular detection. The luminescence color can be tuned by varying the composition of lattice atoms and dopant ions.^{16,45–47} UCNPs also have a narrower width of emission peak (10–20 nm) than that of quantum dots or organic dyes.^{48,49} Combined, these features would allow LUCID to resolve different particles types through simultaneous multicolor detection. Second, we could render UCNPs excitable at longer wavelengths (>1100 nm) wherein the quantum efficiency of semiconductor imagers becomes negligible (due to indirect bandgap of silicon). For example, the excitation wavelength of 1530 nm has been demonstrated when UCNPs were doped with Er and Tm.⁵⁰ Under this condition, LUCID could directly detect particles even with

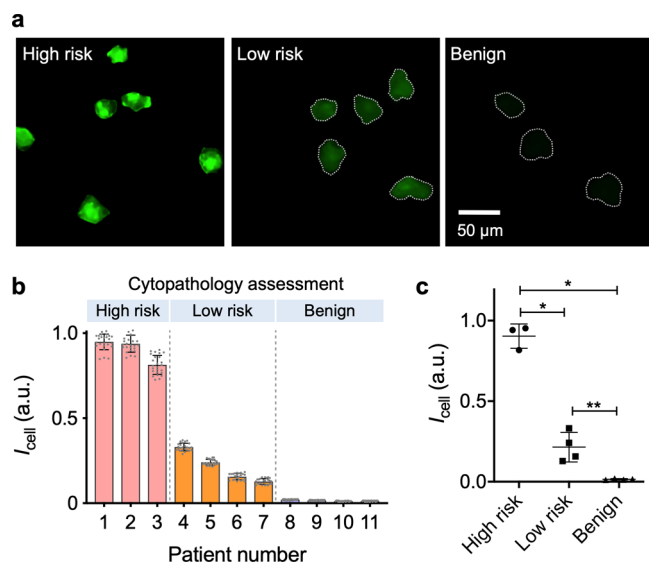


Figure 5. LUCID screening of cervical cancer. (a) Human cervical brushing samples were labeled with a cocktail of UCNPs specific to EpCAM, CD44, and Trop-2. Representative LUCID images from high-risk (cervical intraepithelial neoplasia/CIN 2, 3), low-risk (CIN 1), and benign patient samples are shown. (b) LUCID profiling results from 11 patient samples are displayed in a waterfall plot. For each sample, intensities from 25 labeled cells were measured; risk levels were independently assessed by cytopathology. Increased risk levels were found to be associated with higher LUCID signals. (c) The average cellular intensity was significantly different among high risk, low risk, and benign groups (Sidak's multiple comparisons test): * $p < 0.0001$; ** $p = 0.0094$.

continuous illumination, which will make the system even simpler and easier to use. Third, one could explore the use of phosphorescent particles that exhibit long-lasting (minutes to hours) afterglow. The same time-gated imaging can detect these particles but with longer integration time than that of UCNPs; this would help further enhance the SNR. One technical challenge is establishing new fabrication methods, different from physical grinding of crystals,⁵¹ to synthesize monodisperse, bioconjugatable phosphorescent particles.⁵² These advances in nanoengineering will propel LUCID as an affordable and versatile diagnostic tool to improve and decentralize health care delivery in resource-limited settings.

EXPERIMENTAL SECTION

LUCID System Setup. The excitation light source consisted of a 980 nm laser diode (LDM-0980-002w-35, Roithner LaserTechnik), an aspheric lens (focal length $f = 11$ mm, numerical aperture NA = 0.25; C22MB, Thorlabs), and an objective lens (20 $\times$, Olympus). Two types of signal detectors, a CMOS imager (2592 $\times$ 1944 pixels; DFM 72BUC02-ML; The Image Source) or a smartphone (Galaxy S5, Samsung) with a CMOS imager (1334 $\times$ 750 pixels), were used. A microcontroller unit (Arduino MEGA 2560) was programmed to control the light source and the camera. The laser diode was turned on for 5 ms to excite UCNPs and then turned off. Following 1 ms delay, the camera was on for 10 ms to acquire a luminescence image, and the data were transferred to a computer (~ 70 ms). The acquisition time for a single cycle was thus < 90 ms. We repeated the cycle 20 times. The total illumination time per image was thus ~ 0.1 s, which led to the incident light energy of ~ 20 mJ per sample. Considering the typical sample geometry (350 $\times$ 350 $\times$ 10 μm^3) and the absorption coefficient of water ($\alpha = 5$ m $^{-1}$ at $\lambda = 980$ nm), the energy absorbed by water would be on the order of 10^{-6} J ($= 10$ $\mu\text{m} \times 5$ m $^{-1} \times 20$ mJ), and the resulting increase in the sample temperature is on the order of 0.1 $^{\circ}\text{C}$. The light-source was powered by a battery (9 V D-cell) or through USB connection.

Synthesis of NaGdF₄:Yb/Tm Core UCNPs. We synthesized UCNPs according to the previously reported methods with minor modifications.²³ Here, 0.5 mmol of gadolinium(III) acetate hydrate (99.9%, Aldrich), 0.49 mmol of ytterbium(III) acetate tetrahydrate (99.9%, Aldrich), and 0.01 mmol of thulium(III) acetate hydrate (99.9%, Aldrich) were mixed with 10 mL of oleic acid (technical grade, 90%, Aldrich) and 15 mL of 1-octadecene (technical grade, 90%, Aldrich) in a 100 mL three-neck round-bottom flask. The reaction mixture was heated at 150 $^{\circ}\text{C}$ with stirring for 40 min to remove residual water and oxygen and then cooled to room temperature. A 10 mL methanol solution containing 2.5 mmol of NaOH (98%, Aldrich) and 3.3 mmol of NH₄F (98%, Aldrich) was added to the reaction vessel. The reaction mixture was stirred for 30 min at 50 $^{\circ}\text{C}$ and then heated to 100 $^{\circ}\text{C}$ under vacuum with stirring for 30 min to remove methanol. The temperature was then elevated to 280 $^{\circ}\text{C}$ at the ramping rate of 10 $^{\circ}\text{C}/\text{min}$ and then kept constant for 90 min under Ar. The resulting solution was then cooled to room temperature. The UCNPs were precipitated by adding ethanol and retrieved by centrifugation. The purification procedure was repeated two more times to remove excess surfactant and solvent. The purified UCNPs were dispersible in organic solvents such as hexane and chloroform.

Synthesis of NaGdF₄:Yb/Tm@NaGdF₄:Tb Core-Shell UCNPs. Here, 0.85 mmol of gadolinium(III) acetate hydrate and 0.15 mmol of terbium(III) acetate hydrate (99.9%, Aldrich) were mixed with 10 mL of oleic acid and 15 mL of 1-octadecene in a 100 mL three-neck round-bottom flask. The reaction mixture was heated at 150 $^{\circ}\text{C}$ with stirring for 40 min to remove residual water and oxygen and then cooled to room temperature. A solution of core UCNPs in hexane was injected to the reaction vessel. A 10 mL methanol solution containing 2.5 mmol of NaOH and 3.3 mmol of NH₄F was added into the reaction vessel. The reaction mixture was stirred for 30 min at 50 $^{\circ}\text{C}$ and then heated to 100 $^{\circ}\text{C}$ under vacuum

with stirring for 30 min to remove methanol. Then the reaction mixture was heated to 280 $^{\circ}\text{C}$ at a ramping rate of 10 $^{\circ}\text{C}/\text{min}$, and then kept at that temperature for 90 min under Ar. The resulting solution was then cooled to room temperature. The prepared UCNPs were collected and washed as described above. The shell coating was repeated one more time to form the thick Tb-doped shell.

Synthesis of NaGdF₄:Yb/Tm@NaGdF₄:Tb@NaYF₄ Core-Multishell UCNPs. Here, 1 mmol of yttrium(III) acetate hydrate (99.9%, Aldrich) was mixed with 7.5 mL of oleic acid and 17.5 mL of 1-octadecene in a 100 mL three-neck round-bottom flask. We applied the same reaction condition as those for Tb-doped shell coating, but we used NaGdF₄:Yb/Tm@NaGdF₄:Tb UCNPs as seed particles.

Characterization of UCNPs. The shape, structure, and composition of synthesized nanoparticles were analyzed via transmission electron microscopy (TEM; JEOL 2100, JEOL USA). High resolution TEM (HRTEM) images and energy dispersive X-ray spectroscopy (EDS) were obtained using a JEOL 2100F apparatus equipped with an EDS detector (X-MAX 80T, Oxford). Optical properties of particles were measured by using a fluorescent plate reader (TECAN Safire 2).

Ligand Exchange with Poly(acrylic acid). The surface modification of UCNPs was performed using poly(acrylic acid) (PAA, M_w 1800, Aldrich).²⁵ A volume of 2 mL of UCNPs in chloroform (15 mg/mL) was slowly added to 2 mL of PAA in ethanol (150 mg/mL) with overnight stirring. The solution was centrifuged at 20 000g for 10 min. After washing with ethanol and deionized water for several times, the resulting UCNPs were redispersed in deionized water under sonication.

UCNP-Embedded Beads. A volume of 22 μL of polystyrene beads (Polybead Carboxylate Microspheres, 10 μm , Polysciences) was added into a mixture of butanol (9.5 mL) and chloroform (0.5 mL). After 30 min incubation with stirring, 20 μL of UCNPs in THF (10 mg/mL) was added to the bead solution. After 60 min incubation, the beads were washed using ethanol several times (3000g for 5 min) and then dispersed in deionized water.

Capture Probe Conjugation on the Beads. An amount of 10 mg of streptavidin coated polystyrene beads (SuperAvidin Coated Microspheres, 9.94 μm , Bangs Laboratories) was washed three times (3000g for 3 min) using Tris buffer (20 mM pH 7.5 Tris, 1 mM EDTA, 1 M NaCl, 0.0005% Triton X-100) and then dispersed in 1 mL of Tris buffer. The beads were incubated with 40 μL of biotinylated capture probes (40 nmol, Integrated DNA Technologies) for 60 min at room temperature. After incubation, the beads were washed four times using Tris buffer and dispersed in 1 mL of Tris buffer. The amount of oligonucleotide probes per bead was quantified using the Qubit ssDNA assay kit and Qubit 2.0 fluorometer (Thermo Fisher Scientific). The average number of capture ligands was $\sim 10^6$ per bead.

Conjugation of Oligonucleotide Probes on UCNPs. Volumes of 50 μL of detection probes (50 nmol, Integrated DNA Technologies) and 0.1 mL of EDC (1 mg, 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide hydrochloride, Thermo Fisher Scientific) were added to 0.5 mL of PAA coated UCNPs (~ 30 mg), and they were incubated for 60 min at room temperature. Next, 0.1 mL of EDC (1 mg) was added to the reaction mixture, and then the mixture was incubated overnight. After incubation, the nanoparticles were centrifuged at 20 000g for 10 min. The precipitated UCNPs were redispersed in Tris-Tween 20 (125 mL of 1 M pH 8 Tris, 0.1 g of Tween 20, 375 mL of deionized water) under sonication. The purification process was repeated four times, and UCNPs were dispersed in pH 8 Tris-HCl (20 mM) under sonication. The amount of oligonucleotide probes per nanoparticle (10–20 per particle) was quantified using the Qubit ssDNA assay kit and Qubit 2.0 fluorometer (Thermo Fisher Scientific).

Conjugation of Antibodies on UCNPs. The following antibodies were purchased: anti-HER2 (clone 24D2, Biolegend); anti-EpCAM (clone MAB9601, R&D Systems); anti-CD44 (clone IM7, Biolegend); Trop 2 (clone 162–46.2, Abcam). Volumes of 20 μL of antibody (1 mg/mL) and 0.1 mL of EDC (2 mg/mL) were added to 0.5 mL of PAA coated UCNPs (~ 20 mg), and they were incubated

for 2 h at room temperature. After incubation, the nanoparticles were centrifuged at 20 000g for 10 min. The precipitated UCNPs were redispersed in 1 mL of 1× PBS.

Thrombin Detection. Capture beads were dispersed in Tris-HCl buffer (50 mM pH 7.4 Tris-HCl, 1 mM MgCl₂, 5 mM KCl, 0.1% BSA). A volume of 40 μL of capture beads was incubated with 10 μL of thrombin solution (Human alpha-Thrombin, Haematologic Technologies) for 30 min at room temperature. After incubation, the beads were purified four times using a 5 μm centrifugal filter, and they were dispersed in 40 μL of Tris-HCl buffer. Then 40 μL of beads was incubated with 60 μL of detection UCNPs for 30 min at room temperature. Excess UCNPs were removed via centrifugation (1 min, 60g) using a 5 μm centrifugal filter. Collected beads were dispersed in 50 μL of Tris-HCl buffer.

DNA Detection. A volume of 40 μL of capture beads was diluted with 50 μL of Tris buffer. The beads were incubated with 10 μL of synthetic DNA specific for *S. aureus* (Integrated DNA Technologies) for 30 min at room temperature. After incubation, the beads were purified four times using a 0.45 μm centrifugal filter (94g for 3 min) and were dispersed in 40 μL of Tris buffer. The beads were incubated with 60 μL of detection UCNPs for 30 min at room temperature. Excess UCNPs were removed via centrifugation (1 min, 60g) using a 5 μm centrifugal filter. Beads were then dispersed in 100 μL of Tris-buffered saline (25 mM Tris, 150 mM NaCl).

Bacterial Detection. Four different bacteria species were purchased from the American Type Culture Collection and grown to mid log phase in suitable media: *Staphylococcus aureus* (#25923) in Staphylococcus broth (BD Biosciences); *Klebsiella pneumoniae* (#43816) in trypticase soy broth; *Enterobacter aerogenes* (#13048), *Citrobacter freundii* (#6879) in nutrient broth (BD Biosciences). For RNA extraction, bacteria were centrifuged at 6,000 × g for 10 min and pellets were treated with the preheated Trizol (Life Technologies). The resuspended cells were transferred to 2 mL Safe-Lock tubes (Eppendorf) containing sterilized disruptor beads (0.1 mm, Scientific Industries), and lysed using a vortex mixer. After centrifugation, the supernatant was transferred to a new tube and mixed with an equal volume of ethanol. The mixture was processed by Direct-zol RNA kits (Zymo Research), following manufacturer's protocol. With the extracted RNA samples, the representative single-stranded cDNA was synthesized using random priming with Promega's Reverse Transcription System per manufacturer's protocol. The asymmetric PCR was then carried out in a total volume of 25 μL containing 2.5 μL of cDNA, 0.8 μM excess primer and 0.08 μM limiting primer (Supplementary Table 2), 1× PCR reaction buffer (20 mM Tris-HCl, 20 mM KCl, 5 mM (NH₄)₂SO₄, and 2 mM MgCl₂), 0.2 mM of each dNTP, and 2 U Maxima Hot Start Taq DNA polymerase (Thermo Scientific). The reaction tubes were heated to 94 °C for 2 min, followed by 35 or 40 cycles of 5 s at 94 °C, 15 s at 56 °C, and 15 s at 72 °C, and then a final 10 min extension step at 72 °C.

SYBR Green-Based Quantitative PCR (qPCR). The qPCR was carried out on 7500 Fast Real-time PCR system (Life Technologies) in a total reaction volume of 20 μL containing 2.5 μL of cDNA derived from *in vitro* cultured bacteria (~10⁶ CFU/mL), 0.5 μM of each primer (Supplementary Table 2), and 1× SYBR Select Master mix (Life Technologies). Thermal cycling was then carried out with the following conditions: Initiation (95 °C, 2 min); 40 cycles of denaturation (95 °C, 5 s); annealing (56 °C, 15 s); extension (72 °C, 30 s). The threshold cycle (C_t) values were obtained using the 7500 Fast software. No-template control (NTC) remained undetected, not crossing the established threshold for 40 cycles, which was arbitrarily given a C_t value of 41. The ΔC_t was calculated by subtracting the C_t value of the specimen from the C_t value of NTC.

Cell Preparation for LUCID. A panel of human breast cancer cell lines were purchased (ATCC) and grown in the vendor-recommended media: BT474 (Hybri-Care Medium 46-X with 0.15% NaHCO₃, ATCC); MCF7 (Eagle's minimum essential medium 30-2003 with 0.1% human recombinant insulin, ATCC); HCC1937 (RPMI-1640, Corning). All media were supplemented with 10% heat inactivated fetal bovine serum (Gibco) and 1% penicillin–streptomycin (5000 U/mL, Gibco). Cells were maintained

at 37 °C in a humidified atmosphere containing 5% CO₂. At about 80% confluence, cells were washed, trypsinized, and resuspended to a concentration of ~10⁶ cells/mL in PBS containing 1% BSA. Cells were incubated with 200 μg/mL HER2 conjugated UCNPs for 20 min at room temperature, washed via centrifugation (350g, 5 min), and resuspended in PBS. About 10 μL of cell solution (~10⁵ cells/mL) was placed on a plastic slide for LUCID imaging.

Flow Cytometry. About 10⁵ cells were used for flow cytometry. Cells were suspended in 1% BSA PBS and incubated with 1 μg/mL anti-human HER2 (BioLegend, 324401) antibody for 30 min at room temperature. Cells were then triple washed with 1% BSA PBS, incubated with 1 μg/mL FITC anti-mouse IgG antibody (BioLegend, 406001), and triple washed for flow cytometry measurements. Control samples were prepared and labeled similarly using 1 μg/mL mouse IgG1, κ isotype antibody (BioLegend, 401401) and 1 μg/mL FITC anti-mouse IgG antibody. Fluorescent signal was measured by using a CytoFLEX instrument (Beckman Coulter). The mean fluorescence intensity of 10 000 cells was determined using FlowJo software.

Clinical Samples. The clinical study was approved by the Partners Healthcare Institutional Review Board. We obtained informed consent from adult women referred to the MGH Colposcopy Clinic for cervical biopsy or loop electrosurgical excision procedure (LEEP). One clinical provider (M.A.) performed all cervical procedures and provided excess or otherwise discarded ectocervical or endocervical brushing or biopsy specimens. Study specimens were collected conventionally before loop excision to avoid potential tissue alteration. Cells were rinsed in sterile PBS and concentrated via centrifugation (300g, 5 min). Pelleted cells were treated with collagenase 1 and 2 (5–10 mg/mL; Sigma-Aldrich) at 37 °C, gently agitated and dispersed. Treated cells were washed in PBS and targeted with a cocktail of three UCNPs probes, each specific to EpCAM, CD44, and Trop-2. After 20 min incubation at room temperature, samples were washed via centrifugation (350g, 5 min). Cell pellets were resuspended in PBS (100 μL). We used about 10 μL of samples for LUCID. All experiments were conducted blind to the clinical interpretation of risk.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.9b05634.

Schematic of LUCID device; microscopic and elemental analyses of UCNPs; luminescence lifetime of conventional UCNPs; LUCID assay for soluble molecular targets; comparison of bacteria detection; cell profiling with LUCID; aptamer sequences used for thrombin detection; DNA sequences used for bacterial detection (PDF)

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C.-H.H., Y.I.P., and H.-Y.L. contributed equally to this work. C.-H.H., Y.I.P., C.M.C., R.W., and H.L. designed the research. Y.I.P. synthesized and characterized UCNPs. C.-H.H. built the detection devices. C.-H.H., Y.I.P., H.-Y.L., D.P., and K.S.P. performed bioassays. C.M.C. and R.W. designed the clinical

study. H.-Y.L., M.A., D.P., C.M.C. collected and processed the clinical samples. C.-H.H., Y.I.P., C.M.C, R.W., and H.L. wrote the paper with inputs from all authors.

Notes

The authors declare no competing financial interest.

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