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Probing the nature of upconversion nanocrystals: instrumentation matters

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Probing the nature of nanocrystalline materials such as the surface state, crystal structure, morphology, composition, optical and magnetic characteristics is a crucial step in understanding their chemical and physical performance and in exploring their potential applications. Upconversion nanocrystals have recently attracted remarkable interest due to their unique nonlinear optical properties capable of converting incident near-infrared photons to visible and even ultraviolet emissions. These optical nanomaterials also hold great promise for a broad range of applications spanning from biolabeling to optoelectronic devices. In this review, we overview the instrumentation techniques commonly utilized for the characterization of upconversion nanocrystals. A considerable emphasis is placed on the analytical tools for probing the optical properties of the luminescent nanocrystals. The advantages and limitations of each analytical technique are compared in an effort to provide a general guideline, allowing optimal conditions to be employed for the characterization of such nanocrystals. Parallel efforts are devoted to new strategies that utilize a combination of advanced emerging tools to characterize such nanosized phosphors.

1. Introduction

The rapid development in modern physics and the electronic industry has contributed tremendously to the advances in analytical techniques, allowing characteristics of materials to be probed at the single molecule level with an atomic scale precision. As a typical example, an object with a dimension of 0.1 mm can be recognized by the naked eye, while a conventional optical microscope can reach a resolution of about 200 nm. The resolution can be pushed below 1 nm using...
low-voltage electron microscope and further down to sub-Ångstrom with the help of an aberration corrected scanning transmission electron microscope. With the advent of advanced instruments, most of the measurements nowadays can be completed in a matter of hours (or sometimes even seconds). Recently, the ability of studying materials in situ has enabled a better understanding of fundamental physical and chemical processes during the synthesis.\(^1\)\(^{-3}\) For example, real-time X-ray photoelectron spectroscopy techniques provide necessary insights into the variation in the chemical composition and film thickness of a tungsten oxide thin film grown on a silicon substrate.\(^4\)

Advances in lab instrumentation can exert a profound impact on the understanding of upconversion (UC) materials, characterized by anti-Stokes emission with a spectral shift up to several hundred nanometers.\(^5\) The phenomenon of UC has been extensively investigated in bulk fluoride-based host materials doped with lanthanide ions for more than 50 years.\(^6\)\(^{-9}\) Raman spectroscopy, commonly used to provide quantitative information on the population of a given phonon mode, is an extremely useful tool for the rational design of UC materials. For example, owing to low-energy phonon modes \(<400\text{ cm}^{-1}\) of Na\(\text{YF}_4\),\(^10\)\(^,\)\(^11\) intense UC emission could be generated when embedding a trace amount of lanthanides into the Na\(\text{YF}_4\) host lattice.\(^12\)\(^,\)\(^13\)

Benefitting from the ability of advanced electron microscopes to visualize nano-objects and the rapid development of nanotechnology, lanthanide-doped UC nanocrystals began to emerge in the mid-1990s.\(^14\) In contrast to conventional Stokes-shifting materials such as quantum dots, carbon dots

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and organic dyes, UC nanocrystals hold great promise for fundamental biomedical research because of their unique optical features, including sharp emission bandwidths and long luminescence lifetimes. The excitation by NIR light also eliminates background autofluorescence.\textsuperscript{15–17} Furthermore, high photochemical stability and low cytotoxicity make these nanocrystals ideal as reliable contrast agents for \textit{in vitro} and \textit{in vivo} biological imaging.\textsuperscript{18–20} Since the pioneering works on colloidal UC nanocrystals of LuPO\textsubscript{4}:Yb/Tm\textsuperscript{3+} in 2003\textsuperscript{21} and NaYF\textsubscript{4}:Yb/Er(Tm) in 2004,\textsuperscript{22} enormous success of research has been achieved in preparing UC nanocrystals with controllable size and composition, biocompatible surface coatings, and tunable optical and magnetic properties.\textsuperscript{23–25} Another notable development is the photovoltaic applications of UC nanocrystals as spectral converters to enhance the harvesting of sunlight in the NIR region.\textsuperscript{26–28}

Comprehensive elucidation of the nature of UC nanocrystals is of fundamental importance in interpreting their unique photophysical properties, understanding energy transfer mechanisms, and shedding light on the optimal design of the systems for ultimate applications of these optical nanomaterials. Taking surface coating of nanocrystals as an example, the first thing to note is that the surface ligand employed determines the dispersibility of the nanocrystals in a specific solvent. Surface modification may also exert a considerable influence on cytotoxicity of the nanocrystals.\textsuperscript{19} For instance, poly(acrylic acid)-coated nanocrystals have low to negligible cytotoxicity, while polyethyleneimine- and polyvinylpyrrolidone-protected counterparts present marked cytotoxicity to HeLa and U87MG cells as measured by a standard MTT assay.\textsuperscript{19} Moreover, surface ligands play an important role in altering the luminescence and magnetic properties of lanthanide-doped nanocrystals.\textsuperscript{24,29}

In this review, we focus on state-of-the-art techniques used to characterize UC nanocrystals, from surface structures to their intrinsic properties, namely surface moieties, crystal structures, sizes and morphologies, compositions, optical properties and magnetic characteristics (Fig. 1). In each section, we begin with a brief introduction of the physical principle of each technique, followed by data interpretation related to the properties of UC nanocrystals. The combination of different experimental techniques in the context of synergistic investigations of nanophenomena in UC is also discussed. Driven by the promise of diverse and exciting applications, this field of research offers ultimate challenges in developing instruments for more precise characterization and better understanding of UC nanocrystals at single particle levels.

2. Characterization of surface moieties

The moieties on the surface of lanthanide-doped UC nanocrystals can be generally categorized into two classes: organic species and inorganic materials. The first type is widely utilized through conjugation or functionalization to provide control over the growth kinetics, size, and morphology of the inorganic core. The organic ligands can be further manipulated in the post-synthesis by ligand exchange,\textsuperscript{30} ligand oxidation,\textsuperscript{31} and layer-by-layer assembly,\textsuperscript{32} thereby offering a platform for facile functionalization of the UC nanocrystals. In contrast, the coatings of nanocrystals with inorganic moieties provide a pathway to chemically assemble more complex nanostructures, for example silica-modified core–shell structures for enhanced biocompatibility. Qualitative and quantitative characterization of the surface moieties present on the UC nanocrystals is an essential task after surface modification. A number of instrumentation techniques have been exploited to unveil the surface structures of UC nanocrystals, including IR, NMR and TGA. Note that the characterization of UC nanocrystals by TGA will not be reviewed herein and readers are referred to the literature for more information on this subject.

2.1 IR spectroscopy

The IR technique is a simple but effective analytical tool to characterize ligand binding on the surface of UC nanocrystals. It is in the IR region of the spectrum in which various chemical groups in the molecule can be identified. Ligand molecules are constantly vibrating, and the energy levels corresponding to these motions mostly lie in the IR region, in the wavenumber range of 4000 to 200 cm\textsuperscript{-1}. The combined use of the Fourier transform technique with an interferometer that comes with a Superconducting Quantum Interference Device (SQUID) or a Vibrating Sample Magnetometer (VSM) has been exploited to unveil the surface structures of UC nanocrystals, including IR, NMR and TGA. Note that the characterization of UC nanocrystals by TGA will not be reviewed herein and readers are referred to the literature for more information on this subject.
transmittable through the sample at once. A detector then records the interference pattern which is produced. Moreover, the availability of an attenuated total reflectance technique in conjunction with IR spectroscopy enables direct sampling in the solid or liquid state without further preparation, as well as improved spectral acquisition and reproducibility.33

For illustration, let us consider the case of UC nanocrystals coated with ligands containing a carbonyl (C=O) group. Since the carbonyl group features a very intense absorption band at approximately 1700 cm\(^{-1}\), the validation of UC nanocrystals coated with ligands containing the carbonyl group can be achieved by the IR fingerprinting method. Note that the significant IR spectral bands of representative ligands used in the synthesis of UC nanocrystals are listed in Table 1. Particularly, oleic acids with absorption peaks at 2927 and 2857 cm\(^{-1}\) (stretching vibrations of –CH\(_2\)), 1560 and 1464 cm\(^{-1}\) (stretching vibrations of –COO\(^{-}\)), and 1705 cm\(^{-1}\) (stretching vibration of C=O) have proven valuable for the controlled synthesis of lanthanide-doped nanocrystals. The use of oleic acids with a boiling point of 360 \(^\circ\)C typically favors the preparation of high quality nanocrystals requiring treatment at elevated temperatures in the range of 290 to 310 \(^\circ\)C.63 Importantly, the long alkyl chain of the oleate ligands and the strong binding affinity between the carboxyl group and the lanthanide ions at the surface of the UC nanocrystals render the as-synthesized nanomaterials with excellent dispersibility in non-polar solvents such as chloroform, hexane and cyclohexane. Notably, the oleate ligands can be removed from the particle surface after acid treatment.64 The removal of the oleate ligand can be spectroscopically confirmed by the disappearance of alkene and carboxylate stretching peaks at 3004 and 1564 cm\(^{-1}\), respectively.64

Fourier transform IR spectroscopy can also be applied to the characterization of inorganic coatings made of silicon oxide and carbon on the basis of their characteristic absorptions in the IR region (Si–O–Si: stretching vibration at 1100 cm\(^{-1}\); O–H: stretching vibration at 3320 cm\(^{-1}\); C–OH: stretching vibration at 1000–1300 cm\(^{-1}\)). As a separate note, the amination of silica-coated UC nanocrystals is perhaps the most reliable strategy to introduce amine functional groups for chemical bonding with biomolecules such as protein, DNA and peptide.56–60

It is important to emphasize that the presence of surface ligands may suppress the UC emission of the nanocrystals as opposed to the naked nanocrystals. This phenomenon is known as surface quenching effects, as surface ligands can participate in non-radiative relaxation via stretching vibration of chemical bonds. For example, it was found that the ultraviolet emission intensity of NaGdF\(_4\):Yb/Tm@NaGdF\(_4\) nanocrystals at 290 nm increased substantially after the removal of the oleate ligands.65 The alkyl chain length of the ligands can be correlated well to the surface quenching effect because the trapping of the excitation energy dominates in the ligands containing more methylene units.66 The emission by surface moieties has been reported from surface-capped UC nanocrystals upon excitation at 980 nm as a result of Förster resonance energy transfer between the emissive particle and the ligands at the surface.29,65,67

In some circumstances, IR analysis can be utilized to understand abnormal optical properties of UC nanocrystals by monitoring the chemical reactions between the ligands. For example, Wu and co-workers demonstrated that amidation reaction can

| UC nanocrystals | Surface ligand or coating | Characteristic absorption (cm\(^{-1}\)) | Remarks | Ref. |
|-----------------|---------------------------|---------------------------------------|---------|-----|
| Hydrophobic ligands | LaF\(_3\):Yb(Ho/Tm) | Oleic acid | 2927 and 2857; 1560 and 1464; 1705 | Asymmetric and symmetric stretching vibrations of –CH\(_2\) and –COOH; stretching vibration of C=O | 34–36 |
|                  | NaYF\(_4\):Yb/Er | | | | |
|                  | NaYbF\(_4\):Er | | | | |
|                  | NaYF\(_4\):Yb(Er/Tm) | Oleylamine | 3007; 2926 and 2855; 1098 and 1564 | Stretching vibration of –C–H; asymmetric and symmetric stretching vibrations of –CH\(_2\); stretching and deformation vibration of C–N and –NH\(_2\) | 37, 38 |
|                  | LaF\(_3\):Yb/Er | | | | |
|                  | NaYF\(_4\):Yb/Er | Oleic acid-dioxycycliphosphine | 2940; 1720 | Asymmetric and symmetric stretching vibrations of –CH\(_2\); stretching vibration of C=O | 39, 40 |
| Hydrophilic ligands | YF\(_2\):Yb/Er | PAA\(^a\) | 3491; 1732 and 1422; 1571 and 1461 | Vibration of O=H; stretching vibration of C=O and C–O; asymmetric and symmetric stretching vibrations of –COO\(^{-}\) | 41–43 |
|                  | NaYF\(_4\):Yb(Er/Tm) | PVP\(^b\) | 2958 and 2847; 1651; 1445 | Asymmetrical and symmetrical stretching vibrations of CH\(_2\); stretching vibration of C=O; bending vibration of CH\(_2\) | 30, 44–46 |
|                  | NaYF\(_4\):Yb(Er/Tm) | PVP\(^b\) | 3436; 1638 and 750; 2930 and 2861 | | |
|                  | NaYF\(_4\):Yb(Er/Tm) | PEI\(^c\) | 1374 | Stretching vibration of C=O (N–CO–OH) | 51 |
|                  | BaYF\(_3\):Yb(Ho/Tm) | EDTA\(^d\) | 2927 and 2850; 1610 and 1400 | Asymmetric and symmetric stretching vibrations of –CH\(_2\); stretching vibrations of –COOH | 33, 52–54 |
|                  | NaYF\(_4\):Yb/Er | Citrate | | | |
|                  | NaYF\(_4\):Yb/Er | | | | |
|                  | YVO\(_4\):Er | | | | |
| Inorganic coating | NaYF\(_4\):Yb(Er/Tm) | Amorphous C | 3320, 1000–1300 | Stretching vibration of O=H; vibration of C–OH groups | 55 |
|                  | NaYF\(_4\):Yb(Er/Ho/Tm) | SiO\(_2\) | 1097 | Symmetrical stretching vibration of Si–O–Si | 30, 56–62 |
|                  | NaYF\(_4\):Yb/Er | | | | |
|                  | NaYF\(_4\):Yb/Er | SiO\(_2\)–NH\(_2\) | 3423 and 1637 | Stretching and bending vibrations of N–H | 56–60 |
|                  | BaYF\(_3\):Yb/Er | | | | |

\(^a\) PAA: poly(acrylic acid). \(^b\) PVP: polyvinylpyrrolidone. \(^c\) PEI: polyethylenimine. \(^d\) EDTA: ethylenediaminetetraacetic acid.
enhanced red emission from NaYF₄:Yb/Er (20/2 mol%) nano-
1 or more.70 Nuclei with a spin number of 1/2 can split into
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properties. The spin quantum number of the nuclei can be
having a property called 'spin' which gives them magnetic

spectroscopy. Basically, NMR is concerned with atomic nuclei

2.2 NMR spectroscopy

Perhaps the chemist's favorite structural analytical tool is NMR
spectroscopy. Basically, NMR is concerned with atomic nuclei
having a property called 'spin' which gives them magnetic
properties. The spin quantum number of the nuclei can be
correlated to their shapes. For example, spherical shaped nuclei
have a spin of 1/2, and those with other shapes have a value of
1 or more.70 Nuclei with a spin number of 1/2 can split into
two states, namely +1/2 and −1/2, in the presence of an external
magnetic field. If electromagnetic radiation of radio frequency
is applied to the sample, some of these nuclear magnets
can absorb energy and convert the low spin state (+1/2) to a
higher one (−1/2).

Recently, an NMR technique has been developed as a power-
ful tool for the characterization of organic species immobilized
on inorganic nanoparticles, such as quantum dots and UC
nanocrystals.71–73 The increased line broadening is one of the
problems associated with NMR studies on particle-tethered
ligands due to inhomogeneity in chemical environment and
limited rotational freedom of the ligands. For example, a series
of ¹H NMR chemical shifts from oleic acid-coated NaYF₄
nanocrystals were detected in regions ranging from 0.85 to
5.45 ppm (Fig. 3).71–73 As the size of the particle increases, the
NMR spectrum becomes even more crowded with overlapping
signals - to an extent where the resolution of the peak separa-
tion becomes untenable. The shifting in NMR signals is
another representative characteristic of the ligands anchored
on the surface of UC nanocrystals because the nanoparticle can
significantly shield the magnetic nucleus from the external
field. A typical example is the observation of the chemical shift
in oleic acid from 2.64 to 2.68 ppm due to the presence of the
nanoparticle.74

NMR depends on the magnetic properties of certain nuclei
and subtle alteration of their chemical environment. As a
representative instrument tool for surface ligand characteriza-

tion, NMR is sensitive to the changes in ligands attached on the
surface of UC nanocrystals. For example, ¹H NMR signals of
–HC≡CH– (at 5.34 ppm) and –CH₃ (at 0.85 ppm) of oleic acid
disappeared after chemical transformation to azelaic acid via

Lemieux–von Rudloff oxidation.32 ¹H NMR signals at 5.34 ppm
were also absent when hydroxylation reaction was conducted
on the carbon–carbon double bond.75 When oleate-capped UC
nanocrystals were treated with strong acid to remove surface
ligands, all ¹H NMR signals vanished.64,76,77
NMR is also a reliable tool to monitor the reactions between the ligands to elucidate their “cooperative effect” on size and morphology control, phase transition, and color output of UC nanocrystals. $^1$H NMR signals of NaYF$_4$:Yb/Er (20/2 mol%) nanocrystals synthesized in a mixture of oleic acid and octadecylamine matched well with that of oleamide, evidencing the occurrence of inter-ligand reaction in the synthesis. The generated oleamide promoted the formation of hexagonal-phase NaYF$_4$:Yb/Er (20/0.2 mol%) nanocrystals due to its neutral characteristic. In another parallel experiment, the ligands to elucidate their “cooperative effect” on the phase transition of the resultant UC nanocrystals. Low symmetry of lanthanide-doped hexagonal NaYF$_4$ results in an efficiency of UC up to ten times higher than that of its cubic counterpart. Recently, Wang et al. discovered that the packing manner of lanthanide ions in the crystal lattice can also influence the energy transfer efficiency between Yb$^{3+}$ and Er$^{3+}$. Tetrad clustering of Yb$^{3+}$ ions favors preservation of excitation energy and thus enhances energy transfer from Yb$^{3+}$ to Er$^{3+}$, enabling the generation of an unusual violet emission at 410 nm of Er$^{3+}$ in orthorhombic-phase KYb$_2$F$_7$. In this section, we highlight the utilization of two characterization techniques, based on powder XRD and XAFS spectroscopy, for the structural characterization of UC nanocrystals. The method of electron diffraction will be emphasized in the section of TEM characterization.

### 3.1 Powder XRD

The non-destructive nature and relative ease of sample preparation make powder XRD a widespread technique used for structure determination of UC nanomaterials. The diffraction of scattered X-rays by crystalline materials is depicted in Fig. 4a and b. When the Bragg equation holds, the diffraction pattern will be useful for indexing and identifying the crystal structure. The non-destructive nature and relative ease of sample preparation make powder XRD a widespread technique used for structure determination of UC nanomaterials. The diffraction of scattered X-rays by crystalline materials is depicted in Fig. 4a and b. When the Bragg equation holds, the diffraction pattern will be useful for indexing and identifying the crystal structure.

$$n\lambda = 2d \sin \theta$$  \hspace{1cm} (1)\)

where $n$ is an integer, $\lambda$ is the wavelength of radiation, $d$ is the interplanar spacing, and $\theta$ is the angle of diffraction between the incident ray and the scattering plane (Fig. 4b). In a typical measurement, crystal structure-related information is encoded into diffraction patterns where the X-ray intensity is recorded as a function of the scattering angle equal to $2\theta$. The diffraction angle and intensity are highly sensitive to crystal structures, thereby enabling the identification of crystallinity of UC nanocrystals to be readily identified by comparing the diffraction patterns with well-established databases (standard powder diffraction card of Joint Committee on Powder Diffraction Standards). Strong diffraction peaks are commonly observed from high quality lanthanide-doped UC nanocrystals adopting a hexagonal or cubic phase. These nanocrystals were generally prepared at high reaction temperatures (200 to 300 °C) by various methods, including coprecipitation, hydrothermal treatment, and thermal decomposition.

XRD techniques can also be used to monitor the phase transformation of NaREF$_4$ (RE: rare-earth ion) nanocrystals. For example, the addition of Gd$^{3+}$ ions to NaYF$_4$ precursors renders great control over the phase of the resultant UC nanocrystals as evident by XRD measurements (Fig. 4c and d).
Zeng et al. reported a similar role of Gd$^{3+}$ in the synthesis of NaLuF$_4$:Gd/Yb/Tm nanocrystals and found that the presence of Gd$^{3+}$ can promote the rate of cubic-to-hexagonal phase transformation and facilitate the formation of small-sized UC nanocrystals.\textsuperscript{84} XRD characterization confirmed the reversed phase transformation of NaREF$_4$ (RE: Lu, Gd, Yb) from hexagonal to cubic by insertion of transition metal dopants (e.g., Mn$^{2+}$) into the host lattice.\textsuperscript{85,86} Doping of small-sized alkaline metal ions into RE-based host lattices is also effective for phase control as demonstrated by Dou and Zhang.\textsuperscript{87} In their study, they showed that hexagonal-phase NaYF$_4$ nanoparticles were first converted to cubic ones and further to tetragonal counterparts with increasing dopant concentration of Li$^+$.\textsuperscript{87} Critically, doping of impurities can distort the local symmetry of lanthanide dopants or lead to the variation in energy transfer pathways, thus providing a facile platform to manipulate the optical properties of UC nanocrystals.\textsuperscript{88,89} XRD investigations also provide additional valuable information on UC nanocrystals such as size, lattice strain, and morphology. Appreciable line broadening in diffraction patterns typically indicates a decrease in the size of the particles. Scherrer’s equation (eqn (2)) is usually applied to estimate the average size of UC nanocrystals when the stress in crystallites is not taken into consideration.\textsuperscript{22,71,90}

$$D = \frac{K\lambda}{\beta \cos \theta}$$

where $K$ is the crystallite-shape factor (0.89 for spherical nanoparticles), $\lambda$ is the wavelength of the incident X-ray, $\beta$ is the corrected half width of the diffraction peak and $\theta$ is the diffraction angle. Note that the equation is only applicable to nanocrystals with a size less than 100 nm as the size-dependent line broadening is negligible when the size is over this threshold.\textsuperscript{91} Williamson–Hall theorem (eqn (3)) is required for the estimation of effective particle size and lattice strain when the lattice strain contributes to the effects of diffraction-line broadening as well.

$$\frac{\beta \cos \theta}{\lambda} = \frac{1}{D} + \frac{\eta \sin \theta}{\lambda}$$

where $\beta$ is the full width at half maximum, $\theta$ is the diffraction angle, $\lambda$ is the wavelength of the X-ray, $D$ and $\eta$ are the effective particle size and strain, respectively. The strain and effective size from UC nanocrystals are deduced from the slope and the intercept of a plot of $\beta \cos \theta/\lambda$ against $\sin \theta/\lambda$.\textsuperscript{92} Recently, tensile (positive sign) and compressive strain (negative sign) were observed in LaPO$_4$:Yb/Tm and core–shell LaPO$_4$:Yb/Tm nanoparticles, respectively.\textsuperscript{93} It should be noted that a number of factors, including doping, thermal annealing and shell-coating, can induce lattice strain in the nanosized crystals. Considering that the lattice strain (or local compressive stress) has a marked impact on the optical and electronic properties of colloidal semiconducting nanocrystals and photophysical properties of dye molecules,\textsuperscript{94,95} the study of line broadening by XRD
Techniques can be used to elucidate the correlation between lattice strain and UC processes in lanthanide-doped nanocrystals.86,97

The success of lanthanide doping in UC nanocrystals can be directly measured by the shift of the diffraction lines in the patterns. The position of the diffraction line is strongly sensitive to the d-spacing of scattering atomic planes according to Bragg’s Law. Substantial alteration of d-spacing may be affected by different sized impurities as a result of unit cell shrinkage or expansion (Fig. 4b). For instance, the replacement of Na+ or Y3+ by small-sized Li+ or Mn2+ in cubic NaYF4 host lattice results in shifting of diffraction lines to a higher angle,85,87,88 while big-sized dopants (e.g., Gd3+ and K+) give rise to lower-angle shifting of the diffraction patterns.17,87,98 However, these two opposite effects can offset each other in some cases, generating negligible shifting in the diffraction patterns of doped UC nanocrystals. As revealed by Wang’s group in XRD analysis of Ca2+-doped NaGdF4,99 the expanding effect on cell volume nanocrystals. As revealed by Wang’s group in XRD analysis of Ca2+-doped NaGdF4,99 the expanding effect on cell volume

Anisotropic line broadening observed in the XRD characterization of UC nanocrystals can provide useful information on the morphology of the nanocrystals.100 Theoretically, samples with homogeneous distribution and completely random orientation are required for XRD measurements. Different shaped nanocrystals usually display slightly different diffraction patterns in terms of intensity and anisotropic line broadening.103–105 Spherical and rod-shaped UC nanocrystals typically show enhanced (h00) but diminished (002) reflections, while a reverse trend is observed for hexagonal nanoprisms and nanoplates.103 Abnormal strong diffraction lines can also provide clues on preferential growth directions of the nanoparticle.104 For example, strong diffraction intensity from (100) planes of NaYF4 microcrystals manifests the preferential growth along the [001] crystallographic direction.105,106

XRD techniques can provide compositional mapping of parts of a crystalline species with a weight fraction of larger than 3%. With synchrotron radiation, the sensitivity can be improved down to 0.05% (500 ppm). The low detection limit of XRD clearly makes it difficult to characterize heterostructured or multi-phased nanocrystals in which one component significantly outweighs the others.107–109 Despite the usefulness of the Rietveld refinement for quantitative analysis of NaYF4 nanocrystals with a mixture of cubic and hexagonal phases, the content of the examination component must be larger than 8%.78 This aspect is not trivial and can lead to serious errors if improperly performed.

3.2 XAS

XAS spectroscopy, based on the X-ray photoelectric effect and the wave nature of the electron, can be used to determine local structures around a specific dopant atom. Unlike X-ray diffraction, it places few constraints on the samples and works equally well in amorphous materials, liquids, and polycrystalline solids. The X-ray absorption spectrum is typically classified into two groups: X-ray absorption near-edge spectroscopy (XANES) and extended X-ray absorption fine-structure spectroscopy (EXAFS). XANES is strongly sensitive to formal oxidation state and coordination geometry of the absorbing atom, while the EXAFS is used to provide information about interatomic distances and the coordination number of the absorbing atom.

EXAFS is a powerful technique for determining the chemical state of lanthanide dopants which occur in very low concentration. For example, Song’s group demonstrated that the changes in the Er–O bond length and the coordination number of Er in ZnO:Er nanocrystals, induced by Li+ doping, can be measured by means of EXAFS.110 In a parallel development through use of EXAFS, Liu et al. confirmed that incorporation of Li+ ions into the interstitial sites of Y2O3:Er3+ nanoparticles results in a notable enhancement in Er3+ emission as a result of its reduced symmetric environment.111

As EXAFS focuses on the local structure of a given chemical species irrespective of long range order, it is a versatile tool to correlate the UC emission to the local environment of lanthanide dopants, thereby providing valuable data for rational design of UC nanocrystals with precisely controlled emissions. Chang and co-workers modeled the structure of Y2O3:Er3+ nanocrystals on the basis of EXAFS data and further correlated the results through Judd–Ofelt analysis.112 They found that both approaches match well with each other in predicting optimal doping concentrations of Er3+ for core (5 mol%) and core–shell (2 mol%) nanocrystals. Low limits of detection down to ppm are another notable merit of this technique with synchrotron X-rays as the light source, allowing the UC nanocrystals to be analyzed even at an extremely low dopant concentration. It should be noted that to make the fitting results from XAFS more reliable, the samples should have high homogeneity.

4. Determination of size and morphology

Size and morphology are important factors that affect the optical properties of UC nanocrystals with respect to relative emission intensity (e.g., the green-to-red emission ratio of Er3+), quantum yield, and lifetime.113–115 Both factors also have a significant impact on the performance of UC nanocrystals in nanomedicine concerning the circulation lifetime, cell surface barrier penetration ability, and cell uptake effectiveness. For example, bright UC nanocrystals with diameters smaller than 10 nm are more attractive in bioimaging or biolabeling than their large-sized equivalents.116 In addition, the shape of UC nanocrystals should be taken into consideration when they are exploited as building blocks to fabricate multifunctional solids.117 Morphology-directed assembly into mesoscopic superlattices was observed in the case of NaYF4:Yb/Er (20/2 mol%) nanocrystals or tripodal Gd2O3 nanoplates.103,118 Shape-complementary effects of rhombic GdF3 and tripodal Gd2O3 nanoplates were recently disclosed in the formation of highly ordered superlattices.119 In this section, typical electron microscopes, including TEM, STEM and SEM that use a beam of electrons to construct the specimen image, are introduced and compared for characterizing UC nanocrystals.

The development of electron microscopy techniques has been fueled by their ability to visualize ultra-small objects invisible by conventional light microscopes. Electron microscopy operates
based on the interaction between the electrons and the materials under investigation. A series of signals such as transmitted electrons, backscattered electrons, secondary electrons, Auger electrons and characteristic X-rays are generated, allowing rather comprehensive characterizations of the materials in terms of size, structure, morphology, and composition. As the availability of parallel electron beams in TEM, the use of electron diffraction for studying the crystal structure of UC nanocrystals is also described in this section.

4.1 TEM imaging

TEM can be used as a direct tool to reveal the morphology of lanthanide-doped UC nanocrystals prepared under different synthetic conditions (Fig. 5). Precise growth habits of lanthanide-doped materials under a certain condition are predicable by screening morphology changes in response to varied synthetic conditions. For example, the growth of a conformal anisotropic shell on a nanocrystal core can be modulated by the ligand etching effect and the strain induced by the lattice mismatch between the core and shell materials.120 With the help of TEM characterization, Zhang et al. validated an end-on growth mechanism responsible for the growth of multicolor-banded crystals.121 Another demonstration of utility of TEM for analyzing an epitaxial layer-by-layer growth on NaYF₄ UC nanocrystals was reported by van Veggel's group. 122 In their work, Ostwald ripening dynamics was utilized to yield core–shell structured nanocrystals.

Phase contrast methods of high-resolution imaging offer better spatial resolution than conventional TEM imaging. The diffraction contrast (brightness) from crystalline interfaces due to incident electron scattering difference often contains rows of one-dimensional bands or fringes, which can be used to examine the formation of heterostructures in UC nanocrystals. For example, different components in NaYF₄:Yb/Er-CdSe, NaYF₄:Yb/Tm-Au and NaYF₄:Yb/Er@SiO₂ nanocrystals can be differentiated clearly thanks to the contrast differences in TEM images.61,123–125 For NaYF₄@NaGdF₄ nanoparticles, the structure and chemical composition at the core–shell interface can hardly be distinguished by this imaging technique as the core and shell components have similarcapability of scattering incident electrons. Under these circumstances, the formation of core–shell nanostructures via seed-mediated growth methods can be indirectly identified by measuring the average size of the nanocrystals, the enhancement in emission intensity, and the change in the emission lifetime and quantum yield.126,127

Under a high-resolution imaging mode, the crystallographic structures of UC nanocrystals, including crystalline defects and crystallite domains, can be inspected at an atomic level (Fig. 6a and b).65,126 Interface-specific inspection for UC nanocrystal-based heterodimers provides important information on the growth mechanism, laying the foundation for the fabrication of multimodal imaging agents.107,128,129 Much detailed analysis of UC nanocrystals is often performed with a phase-amplitude diagram – a graphical construction of a complex Fourier transform.82 Typically, a refined-HRTEM image is utilized to construct a corresponding symmetry-imposed image. The amplitude and
phase are extracted from the lattice position of the Fourier transform and analyzed against the different plane groups using professional image processing programs. Using this approach, quantitative analysis of K, Yb and F atoms in the KYb₂F₇:Er lattice can be obtained as shown in Fig. 6c.⁸² It is worth noting that TEM can also be operated in the scanning mode, known as STEM, to perform point-by-point scanning across samples using a focused electron probe. The STEM technique offers impressive imaging resolution down to sub-Ångstrom under an accelerating voltage of 300 kV, thereby enabling to resolve single atoms as demonstrated in a NaGdF₄:Yb/Tm@NaGdF₄:Tb core–shell nanocrystal (Fig. 6d).⁶⁵

A unique feature of UC nanocrystals is their relatively low thermal stability under electron beam irradiation, thus posing a challenge in acquiring high quality HRTEM images.¹³⁰ Yan and co-workers irradiated a single NaYF₄:Yb/Er nanoparticle with a spark of an electron beam for an extended time period and observed in situ solid-to-hollow transformation of the particle (Fig. 7).¹³¹ Gao and co-workers attributed this phase transition to a cooperative result of knock-on effects, specific lattice strain and low surface energy associated with NaREF₄ nanomaterials.¹³² Johnson et al. recently highlighted that the stability of coreshell UC nanocrystals on high-dose irradiation is closely related to the interfacial strain.¹³³ Tensile-force-shelled UC nanocrystals, such as NaYF₄@NaTmF₄, exhibited a deleterious stability as opposed to their compress-shelled equivalents (e.g., NaYF₄@NaDyF₄). These findings may provide an intriguing strategy to pattern lanthanide-doped nanocrystals by electron beam manipulation. One promising way of minimizing the overheating effect is to use cryogenic TEM for reliable sample analysis as suggested by Zhao and co-workers.¹³⁴

As an added benefit, TEM provides imaging capability of SAED on individual UC nanocrystals by taking advantage of electro-magnetic lens and physical apertures. Phase-specific patterns of dots in single-crystal structures have been observed in the SAED measurement of Gd³⁺-doped NaYF₄, Er³⁺-doped NaYbF₄, NaYF₄:Yb/Er and NaYF₄:Yb/Ho/Tm nanocrystals.¹⁷,¹²⁸,¹²⁹,¹³⁵,¹³⁶ Moreover, combining the results of HRTEM with SAED data performed on single nanocrystals can provide valuable information on the growth direction of NaYF₄:Yb/Er (18/2 mol%) nanowires.¹³⁷

A powder diffraction pattern, similar to the case of powder XRD, can be generated when a large number of nanocrystals are illuminated by an electron beam (Fig. 8a and b).²⁶,¹¹⁶,¹³⁸–¹⁴⁰ The diameter (D) of the ring in the powder pattern can be derived from eqn (4), where d refers to lattice spacing of a specific scattering atomic plane, λ is the wavelength of the electron beam, and L is the effective camera length of the electron microscope.

\[
d = \frac{2\lambda L}{D}
\]

A number of factors, such as doping or shell coating, principally give rise to changes in the d-spacing (Fig. 8, table).

Fig. 7 Morphologic evolution of a hexagonal-phase NaYF₄:Yb/Er (20/2 mol%) nanocrystal under electron beam irradiation at different time intervals. (a–f) 0, 15, 30, 60, 90 and 120 s, respectively. (Reprinted with permission from ref. 131. Copyright 2009, Wiley-VCH Verlag GmbH & Co. KGaA.)

Fig. 8 Characterization of UC nanocrystals by SAED. (a) TEM image of NaYF₄:Yb/Er (20/2 mol%)@NaGdF₄ nanocrystals and (b) corresponding powder patterns of the nanocrystals by SAED characterization. (*) Comparison of observed relative ring spacing (d-spacing) (OBS) and stimulated values on the basis of bulk NaYF₄ (SIM₁) and NaGdF₄ (SIM₂) crystals. The deviation between the experimental data (NaYF₄:Yb/Er@NaGdF₄ nanocrystals) and SIM (bulk NaGdF₄ and NaYF₄ crystals) values for each set of miller indices (hkl values) were quantitative evaluated in terms of σ value. \[\sigma_{1} = (S\text{IM}_{1} - \text{OBS})/\text{OBS}; \sigma_{2} = (S\text{IM}_{2} - \text{OBS})/\text{OBS} \] (Reprinted with permission from ref. 134. Copyright 2012. American Chemical Society.)
It should be noted that the detection sensitivity of SAED is lower than that of XRD because of errors possibly induced by specimen defects, the aberrations of a given electromagnetic lens, and limitations in connection with the recording systems.

SAED is an extremely useful technique for providing information on the packing order of the nanoparticles upon aggregation. Murray and co-workers reported the observation of single crystal-like diffraction patterns from superlattices of anisotropic UC nanocrystals. The single crystal-like diffraction patterns implies highly ordered packing in position and orientation of the nanocrystal building blocks. For SAED measurements, it is worth noting that the specimen under examination should be thin (typically less than 100 nm) to reduce the inelastic scattering of the incident electron beam, allowing reflection spots to be generated in the diffraction patterns.

4.2 STEM imaging

Apart from high imaging resolution, STEM in a high-angle annular dark-field (HAADF) imaging mode is a terrific technique that can be used to distinguish the interfacial region of a core–shell UC nanocrystal. This capability stems from the fact that heavy atoms tend to generate high-angle scattered electrons and the contrast difference obtained by HAADF-STEM imaging of different components in a nanocrystal is proportional to the square of $Z$ ($Z =$ atomic number). Different layers in a variety of core–shell or multi-layer core–shell UC nanocrystals have been characterized by the HAADF-STEM imaging technique, including NaYF$_4$@NaGdF$_4$, NaGdF$_3$Yb/Ca/Er@NaYF$_4$, NaYbF$_4$Tm@CaF$_2$, and NaGdF$_3$Nd@NaYF$_4$@NaGdF$_4$Nd/Yb/Er (Fig. 9).

Despite a number of excellent capabilities, the STEM imaging technique has inherent limitations and problems, some of which are associated with the low thermal stability of lanthanide-doped nanocrystals in response to electron beam irradiation. Similar to the mitigation measure adopted by TEM, the damage to the nanocrystals in STEM mode can be largely reduced by imaging the nanocrystals at a low temperature of 96 K. Notably, the scanning mode of STEM is attractive in analyzing the spatial distribution of elements in a nanocrystal which will be described in the section of composition analysis.

4.3 SEM imaging

SEM is a convenient method for high resolution imaging of surfaces. The advantages of SEM over STEM include great depth of field and 3D rendering opportunity albeit their similarity in the operating mechanism. SEM utilizes secondary electrons, or backscattered electrons or a mixture of both types of electrons for imaging. As the signals can be generated from the surface layer of the specimen from several to 100 nm in depth contingent on acceleration voltage, SEM provides a fertile ground for applications in characterizing the surface characteristics of small-sized UC crystals, such as flower-like β-NaYF$_4$ hexagonal disks and NaYbF$_4$ microtubes (Fig. 10a and b). With regard to drawbacks, the prevalent use of SEM under normal operating conditions for imaging small-sized UC nanocrystals is hindered by the charging effect due to low conductivity of the nanocrystals (Fig. 10c). Scanning faults and image artifacts resulting from the charging effect can be effectively minimized by coating the nanocrystals with a thin layer of a noble metal or carbon. Yan and co-workers found that by properly adjusting the acceleration voltage and imaging distance, the resolution of SEM could reach 20–30 nm for NaYF$_4$Yb/Er (20/2 mol%) nanoparticles without the surface coating (Fig. 10d).

Fig. 9 HAADF-STEM for effectively differentiating the core and shell layers of UC nanocrystals. (a) NaYF$_4$@NaGdF$_4$. (b) NaGdF$_3$Yb/Ca/Er@NaYF$_4$. (c) NaYbF$_4$Tm@CaF$_2$. (d) NaGdF$_3$Nd@NaYF$_4$@NaGdF$_4$Nd/Yb/Er. (Reprinted with permission from ref. 99, 127, 143, 144. Copyright 2011, 2013, 2012 and 2013, American Chemical Society, Royal Society of Chemistry and Nature Publishing Group.)

Fig. 10 SEM characterization of lanthanide-doped UC crystals. (a) Flower-patterned hexagonal plates of NaYF$_4$. (b) NaYbF$_4$Tm (1.0 mol%) microtubes. (c) NaYbF$_4$ microplates (unpublished result). (d) NaYF$_4$Yb/Er (20/2 mol%) nanocrystal assemblies. (Reprinted with permission from ref. 106, 145 and 147. Copyright 2007, 2010, 2011 and 2009, Wiley-VCH Verlag GmbH & Co. KGaA, American Chemical Society and Royal Society of Chemistry.)
5. Composition determination

The concentration of the dopant in UC nanocrystals plays a very important role in determining the optical characteristics of the nanocrystals as non-radiative de-population of activators highly depends on the level of doping. Consequently, UC nanocrystals with desirable optical features are achievable by deliberately optimizing doping concentration of sensitizers (Nd\(^{3+}\), Yb\(^{3+}\)) and activators (Er\(^{3+}\), Ho\(^{3+}\), Tm\(^{3+}\)). Presently, the dopant concentration of UC nanocrystals is typically estimated from the amount of the lanthanide precursors added to the solution, assuming that all the lanthanides are homogeneously doped into the nanocrystals after the reaction. However, one would expect a small discrepancy between predicted and observed values for the dopant concentration, partly introduced by a side reaction of the lanthanide precursors with the ligands (e.g., oleic acid). For example, Yan and co-workers found that the composition of NaYF\(_4\):Yb/Er nanocrystals varied with the reaction time via thermal decomposition of the lanthanide trifluoroacetate precursor. Yi and colleagues reported a similar phenomenon when preparing NaYF\(_4\):Yb/Er nanocrystals in the presence of ethylenediaminetetraacetic acid by a hydrothermal method. They ascribed the change in composition to different coordination abilities of lanthanides to EDTA, thus affecting the supersaturation and precipitation processes key to the formation of nanocrystals.

In this section, we discuss instrumental techniques used to measure doping concentration of lanthanide ions in UC nanocrystals. Three general microanalytical techniques, EDS, EELS, and XPS, will be first reviewed for their use in characterizing UC nanocrystals (Table 2). Then, we intend to highlight the application of ICP-MS and ICP-AES techniques for acquiring the dopant concentration of UC nanocrystals in bulk form.

### 5.1 EDS

EDS is an X-ray microanalytical technique that can provide information on the elemental composition of a sample upon bombarding of high energy electron beam. As a separate merit to TEM imaging, EDS is commonly used for elemental mapping of UC nanocrystals. The energies of the characteristic X-rays allow the elements making up the nanocrystal sample to be identified, while the intensities of the X-ray peaks allow the quantification of dopant concentrations. For example, a wide variety of lanthanides, such as Y, Gd, Tb, Yb, and Er, are detectable by EDS analysis of single nanoparticles (Fig. 11a). EDS in line-scanning mode is particularly useful in the characterization of core–shell UC nanocrystals. As demonstrated by van Veggel and co-workers, the core–shell structure of NaYF\(_4\)@NaGdF\(_4\) nanoparticles could be verified using EDS technique on the basis of the spatial distribution of Y\(^{3+}\) and Gd\(^{3+}\). This result is in good agreement with that obtained from HAADF-STEM (Fig. 11b). The changes in the relative signal intensity of lanthanides (e.g., Y\(^{3+}\)) along the scanned line can also provide supporting evidence for the generation of hollow nanostructures. 2D scanning of EDS can give an overall estimation of lanthanide distribution within the nanostructures from a projection plane, enabling to characterize UC nanocrystal-based heterostructures and elucidate crystal growth mechanism. For instance, different parts of

### Table 2 Comparison of EDS, EELS and XPS used for characterizing the composition of UC nanocrystals

| Technique | Incident illumination | Signals | Spatial resolution (nm) | Detection limit (atom%) | Single-particle characterization | Core–shell characterization | Ref. |
|-----------|----------------------|---------|------------------------|------------------------|------------------------------|---------------------------|------|
| EDS\(^a\) | Electron beam        | X-ray   | 1–10                   | 0.1                    | Yes                          | Yes                       | 65, 156 and 157 |
| EELS      | Electron beam        | Electron| 0.1–1                  | 0.05                   | Yes                          | Yes                       | 65, 134, 162 and 163 |
| XPS       | X-ray                | Electron| 5000–30 000           | 0.01–0.5               | No                           | Yes\(^b\)                | 64, 124 and 164–168 |

\(^a\) The parameters of EDS characterization are controlled by TEM imaging. Spatial resolution and detection limit by SEM are much lower than achievable by TEM. \(^b\) The shell thickness should be less than 10 nm.
NaYF₄(Yb³⁺/Er³⁺)/C-doped TiO₂ composite materials have been fully characterized using this technique. Detailed elemental distributions of Lu, Yb and Y in α-NaLuF₄@β-NaYF₄ and α-NaYF₄@β-NaLuF₄ core–shell nanocrystals, prepared by a cation-exchange reaction, have been visualized from respective core and shell layers by the 2D EDS scanning technique.

It has been challenging for EDS to quantitatively analyze the doping concentration of activators although its detection limit reaches 0.1 at% under optimized conditions (Table 2). For example, the detection of Tm³⁺ in NaYF₄:Yb/Tm (20/0.2 mol%) nanocrystals by EDS is extremely difficult. Moreover, impurities (e.g., O and C) may easily interfere with the determination of the dopant ions. Additionally, samples containing many closely spaced X-ray lines from lanthanides, such as lanthanum and cerium, may also add a constraint to the quantitative mapping of the dopants.

5.2 EELS

By inspecting the energy loss of incident electron beam, EELS is another representative technique for qualitative and quantitative analysis of dopants in UC nanocrystals. The use of an angle-limiting aperture can limit the size of the volume element generating the energy-loss signal. As a result, the spatial resolution can be pushed down to 0.1–1 nm by the EELS technique, a resolution that is much higher than obtainable by EDS (approximately 1–10 nm) (Table 2). The differentiation of core–shell UC nanocrystals is easily achievable by the EELS analysis. For example, using such a high resolution technique Y³⁺ and Gd³⁺ elemental signals could be detected from the core and shell regions in a NaYF₄@NaGdF₄ nanocrystal (Fig. 12).²,⁴,₁₆₂ Analogous to EDS, EELS in 1D or 2D scanning mode can also produce solid proof distributions of Lu, Yb and Y in a nanocrystal using such a high resolution technique Y³⁺ and Gd³⁺ elemental analysis. For example, the replacement of Y³⁺ in NaYF₄ by a trace amount of Gd³⁺ can be readily confirmed by XPS measurement but not other techniques, including XRD and EDS.³⁸,₁₆₅

Depth-dependent composition measurement by XPS provides an alternative method to study the dopant distribution in UC nanocrystals. By using synchrotron radiation, van Veggel and co-workers reported precise control over the penetration depth of X-rays in NaGdF₄ nanoparticles.⁶,₄,₁₂,₄,₁₇₁ They found that the distribution of Y³⁺, Tb³⁺ or Nd³⁺ in the NaGdF₄ nanoparticles is inhomogeneous. In a subsequent work, the researchers reported that the cation exchange competes with precipitation during the synthesis of core–shell nanocrystals. The resultant nanocrystals, as characterized by XPS, showed a concentration gradient of lanthanides or complex alloy structures.₁₆₂ They further extended this approach to characterizing core–shell UC nanocrystals by analyzing the relative signal intensity of lanthanides as a function of the photoelectron kinetic energy.₁₆₈ The gradually increased signal ratio of Y³⁺-to-Gd³⁺ along the penetration depth of a specimen revealed that the specimen is composed of NaGdF₄@NaYF₄ core–shell nanocrystals (Fig. 13).₁₆₈ Unlike EDS and EELS techniques offering high spatial resolution, the spatial resolution of XPS is typically within the range of 5–30 μm (Table 2).

5.3 XPS

A focused X-ray beam is also suitable as a light source to generate electrons for qualitatively or quantitatively probing surface composition of solid matters, a technique known as XPS. The amount of electrons and corresponding kinetic energies on X-ray irradiation is recorded by an electron energy analyzer for elemental analysis. Each element in a specific chemical environment has a characteristic binding energy (\(E_{\text{binding}}\)) (eqn (5)).

\[
E_{\text{binding}} = E_{\text{photon}} - (E_{\text{kinetic}} + \phi)
\]

where \(E_{\text{photon}}\) is the energy of initial X-rays, \(E_{\text{kinetic}}\) represents kinetic energy of emitted electrons, and \(\phi\) is an instrument related parameter.

Owing to the weak escape capability of ejected electrons, XPS is only limited to characterize surface composition of UC nanocrystals with a thickness of less than 10 nm. The high sensitivity (down to ppm) of this technique, combined with the ability to measure the surface structure, makes this technique ideal for probing subtle variations on the surface of UC nanocrystals.⁶,₄,₁₂,₄,₁₇₁ For example, the replacement of Y³⁺ in NaYF₄ by a trace amount of Gd³⁺ can be readily confirmed by XPS measurement but not other techniques, including XRD and EDS.³⁸,₁₆₅

The aforementioned three techniques are only applicable to micro-domain analysis within single or a small collection of nanocrystals. More reliable analysis that provides information on the average dopant concentration of lanthanides in UC nanocrystals is merely feasible by ICP-MS and ICP-AES measurements. Although the limit of detection for ICP-MS is about one order of magnitude higher than ICP-AES, both techniques...
can help identify the amount and type of elements present in lanthanide-doped UC nanocrystals. Both ICP-MS and ICP-AES techniques have been widely applied to probing the average concentrations of dopants in UC nanocrystals after chemical dissolution. It should be pointed out that the analytical results always deviate from the stoichiometric values used for the nanocrystal synthesis. In addition to the error caused by the imperfection of measuring instruments, different reactivity of lanthanide ions with ligands or with Na⁺ and F⁻ may also contribute to the deviation. Using ICP-MS, Zhou et al. found that azelaic acid-capped NaGdF₄:Yb/Er/Tm nanoparticles are first uptaken by liver and spleen, shortly after the injection of the particles (40 min), followed by heart, lung and kidney.

Combined with laser ablation, the ICP-MS/AES analytical technique could be used for the bulk sampling of solid UC nanocrystals, in addition to being used for the analysis of solutions. An intrinsic limitation to ICP-MS is the possible spectroscopic interference by Re⁺ and ReO⁺ species formed during the step of ion generation. For ICP-AES measurement, the challenge lies in how to extract effective signals from background emissions.

### 6. Measurement of optical properties

Optical characteristics of lanthanide-doped UC nanocrystals are the most fascinating part of their properties. Anti-Stokes emissions of lanthanide activators have been achieved through effective energy transfer between sensitizers (Table 3) and activators (Table 4) or by excited state absorption of the activators. Unlike the vast majority of transition metal counterparts with only one emitting excited states, lanthanide dopants feature a series of f–f excited states, making them particularly suitable for use in UC materials. Due to the parity-forbidden 4f–4f transition, lanthanide-doped UC nanocrystals have long lifetimes of emission up to 20 ms and relatively small absorption cross-sections. For characterization of optical properties of UC nanocrystals, we start from the introduction of excitation source selection, including wavelength, power density and laser types (continuous-wave laser or pulse laser), then move to the measurement of emission, quantum yield and lifetime, followed by the discussion on the challenges in analyzing energy transfer efficiency between UC nanocrystals and other optical systems. A considerable effort is devoted to describing the integrated techniques for characterizing UC nanocrystals on the single-particle level. In the last part of this topic, we briefly summarize recent advances in instrumentation for in vitro and in vivo imaging.

#### 6.1 Choosing appropriate excitation wavelength

A new direction of research in the field of UC is to broaden the range of wavelengths used for excitation. Previously, the excitation of 980 nm was oftentimes used because it perfectly matches the absorption band of Yb³⁺, typically doped in the UC nanocrystals as a sensitizer. In addition, the use of the Yb³⁺ sensitizer enables to achieve efficient energy transfer due to the effects of resonance – a process that occurs when the ²F₇/₂ → ²F₅/₂ transition of Yb³⁺ matches many f–f transitions of upconverting lanthanide ions (Er³⁺, Tm³⁺, and Ho³⁺). However, 980 nm laser sources for biological imaging often cause strong water absorption and sample overheating. To expand the excitation beyond the 980 nm laser, an effective solution is to use Nd³⁺ ions as the sensitizer for photon UC under 800 nm

![Composition characterization of UC nanocrystals by XPS technique. (a) Signals of yttrium 3d and gadolinium 4d core levels from NaGdF₄@NaYF₄ nanocrystals before the subtraction of the background (hν = ca. 450 eV). (b) Intensity ratio of yttrium (3d) to gadolinium (4d) core levels as a function of photoelectron kinetic energy. (Reprinted with permission from ref. 168. Copyright 2009, American Chemical Society.)](image)

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**Table 3** Traditional sensitizers for pumping UC emissions

| Sensitizer | Absorption band (nm) | Excitation transition | Excitation wavelength (nm) | Ref. |
|------------|----------------------|-----------------------|---------------------------|------|
| Nd³⁺       | 860, 800, 740        | ²F₇/₂ → ²F₅/₂, ⁴F₅/₂, ⁴F₇/₂/₂H₁₂/₂, ⁴S₃/₂/²F₅/₂ | 808, 800, 795 | 163 and 175–178 |
| Yb³⁺       | 980                  | ²F₇/₂ → ²F₅/₂        | 915, 980                | 149 and 179 |
| Er³⁺       | 1500, 970, 800       | ²F₇/₂ → ²F₅/₂, ²F₇/₂ → ²F₅/₂ | 800, 980, 1490, 1530, 1560 | 180–182 |
| Ho³⁺       | 1140–1250            | ²F₇/₂ → ²F₅/₂, ²F₅/₂ → ²F₇/₂, ²F₅/₂ → ²F₅/₂ | 1560 | 182 |

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excitation, as Nd$^{3+}$ features a sharp absorption band centered around 800 nm. Conversely, the excitation can be shifted to a wavelength much longer than 980 nm, as demonstrated by Prasad and co-workers in LiYF$_4$:Er (10 mol%) colloidal solution. In their work, they observed an intense UC emission from the nanoparticle solution upon laser excitation at 1490 nm. In a parallel development, Wang and co-workers reported the fabrication of multilayer NaGdF$_4$:Er@NaGdF$_4$Ho@NaGdF$_4$ nanocrystals excitable by dual wavelengths, 1160 nm for Ho$^{3+}$ and 1500 nm for Er$^{3+}$. The strong UV-visible emissions of these nanocrystals upon NIR excitation potentially provide a much needed solution for reducing transmission loss in crystalline silicon solar cells.

6.2 Excitation by continuous-wave (CW) laser

As photon UC is a representative non-linear optical process, the luminescence properties of UC nanocrystals are markedly dependent on the excitation source. In this section, we focus on the steady-state UC emissions of UC nanocrystals under excitation by CW lasers within different ranges of power density: 1–10² W cm$^{-2}$ (low), 10²–10⁴ W cm$^{-2}$ (medium) and 10⁴–10⁶ W cm$^{-2}$ (high).

6.2.1 Low excitation power density ($<$ 10² W cm$^{-2}$). Quantum dots and organic dyes generally require relatively high excitation power densities of 10⁴ W cm$^{-2}$ and 10⁶–10⁹ W cm$^{-2}$, respectively, to generate anti-Stokes emissions. This situation is sharply contrasted by the optical behavior of lanthanide-doped nanocrystals. Due to the long lifetime of lanthanides and the existence of many emitting excited states, anti-Stokes emission can be realized from the lanthanide-doped nanocrystals with much lower excitation power density ($<$ 10 W cm$^{-2}$). Under this circumstance, the UC intensity of the emission band is proportional to $n$ power of the NIR excitation power $I_p$ (eqn (6)).

$$I_{UC} \propto I_p^n$$

where $n$ represents the number of NIR photons absorbed per photon emitted in the UV-to-visible region. For this reason, the number of NIR photons involved in the UC process can be calculated from a function of emission intensity against excitation density. In most cases, both red and green emissions of Er$^{3+}$ are confirmed as a result of a two-photon UC process. Note that with increasing power density, the saturation of excitation is usually observed from lanthanide-doped nanocrystals, illustrating the cooperative effect of sensitizers and activators on the UC process. The dependence of emission on excitation power density offers an interesting method to modulate color output of the UC nanocrystals without the need for modifying the composition of the nanocrystals. Due to the effects of thermal annealing and laser irradiation, giant enhancements up to 2 to 3 orders of magnitude in emission intensity of NaYF$_4$:Yb/Er nanocrystals have been reported by two independent research groups.

6.2.2 Medium excitation power density (10²–10⁶ W cm$^{-2}$). A medium excitation power density in the range of 10²–10⁶ W cm$^{-2}$ is commonly required for imaging UC nanocrystals using a wide-field fluorescence microscope that mainly contains a pumping source (980 nm laser), an objective, filters, and dichroic mirrors, as illustrated in Fig. 14a. This design allows simultaneous excitation of all UC nanocrystals exposed in the optical path.

UC nanocrystals or their aggregates with size larger than the diffraction limit of light (~200 nm) can be clearly recognized by a wide-field optical microscope (Fig. 14b and c). Direct imaging of a single UC nanocrystal by a wide-field microscope is generally challenging but can be made possible with the assistance of other imaging techniques such as STEM or AFM. For example, Park and co-workers first demonstrated the measurement of UC luminescence of a single cubic-phase NaGdF$_4$:Yb/Er@NaGdF$_4$ nanoparticle by a two-step procedure. The first step is to localize the nanoparticle with precision via AFM, and the following step involves spectroscopic study of the nanoparticle using a wide-field fluorescence microscope (Fig. 14d-f). The authors provided concrete evidence that lanthanide-doped nanocrystals have exceptionally high photostability against laser beam illumination (100–170 W cm$^{-2}$) for 4 h. In a recent work by the same group, they suggested that the high photostability against photobleaching is a general characteristic of the UC nanocrystals, regardless of whether different host materials or core–shell strategies are implemented.

6.2.3 High excitation power density (10⁴–10⁶ W cm$^{-2}$). A high power density of 10⁶ W cm$^{-2}$ can be generated by focusing laser beam into a tiny volume with a diameter of less than 1 µm. The focused laser beam enables pumping of UC nanocrystals under extremely high excitation power density. In 2009, a team led by Cohen and Schuck investigated the optical properties of a hexagonal-phase NaYF$_4$:Yb/Er (20 mol%) nanocrystal (~27 nm) using a confocal setup equipped with a CW laser (Fig. 15a). In 2010, they pushed the size limit of single-particle analysis down to sub-10 nm using core–shell nanocrystals. In both
cases, superior photophysical stability of UC nanocrystals was further verified under high power density excitation up to $3 \times 10^6 \text{ W cm}^{-2}$ (Fig. 15b and c).

Polarized energy transfer UC in a single hexagonal NaYF$_4$:Yb/Tm (18/2 mol%) microrod was recently demonstrated by Zhou et al., who exploited confocal microscope imaging coupled with a linearly polarized laser. The authors argued that the polarization anisotropy, induced by intra-ionic transition and the breaking of crystal local site symmetry, is responsible for the polarized UC emissions.$^{199}$ By using excitation with intense power density (up to $2.5 \times 10^6 \text{ W cm}^{-2}$), Jin and co-workers observed a suppressed concentration-quenching effect in UC nanocrystals containing a high concentration of Tm$^{3+}$ ($8 \text{ mol%}$).$^{200}$ Similar results were observed by Gargas et al. from ultrasmall NaYF$_4$ nanoparticles ($5 \text{ nm}$) co-doped with Yb$^{3+}$/Er$^{3+}$ (20/20 mol%).$^{201}$ The authors found that the brightness of these nanoparticles can surpass the conventional nanoparticles (β-NaYF$_4$ with 20% Yb$^{3+}$ and 2% Er$^{3+}$) when the power density reaches $\sim 3.0 \times 10^5 \text{ W cm}^{-2}$ (Fig. 15d–g). Such ultra-small, bright UC nanocrystals may find use in a range of applications, particularly in the area of single-molecule detection. An additional benefit of high power density excitation lies in its ability to trap single UC nanocrystals and perform in situ optical characterization.$^{202}$

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**Fig. 14** Characterization of UC nanocrystals under excitation at a medium power density. (a) Schematic configuration of a wide-field luminescence microscope. (b) Wide-field luminescence images of multicolored barcodes with an average length of 2 μm. (c) Wide-field luminescence image of UC nanocrystal-decorated polystyrene microspheres. (d) Luminescence image of NaGdF$_4$:Yb/Er (20/2 mol%)@NaGdF$_4$ core–shell nanocrystals and corresponding AFM characterization. (e) Size measurement of individual core–shell nanocrystals shown in (d). (f) The corresponding photophysical stability of the core–shell nanoparticles as shown in e on 980 nm excitation ($100–170 \text{ W cm}^{-2}$). (Reprinted with permission from ref. 65, 121 and 196. Copyright 2011, 2014 and 2009, Nature Publishing Group, American Chemical Society and Wiley-VCH Verlag GmbH & Co. KGaA.)

**Fig. 15** Characterization of UC nanocrystals under excitation at a high power density. (a) Confocal UC luminescence imaging of UC nanocrystals on a silicon nitride membrane (inset: corresponding SEM image in transmission mode). (b) Time-dependent emission intensity of a single UC nanocrystal under continuous laser illumination with a power density of about $3 \times 10^6 \text{ W cm}^{-2}$. (c) Zoom-in time trace and histogram of emission intensity of a UC nanocrystal showing a non-blinking characteristic. (d) Luminescence intensity of a single nanocrystal comprising NaYF$_4$:Yb/Er (20/20 mol%, blue circles) and NaYF$_4$:Yb/Er (20/2 mol%, red circles), respectively, plotted as a function of excitation intensity. Note that the inset shows zoom-in of the luminescence intensity cross-over region measured for the two different nanocrystals. (e–g) Confocal luminescence images taken at points shown in (d). The images were collected at increasing excitation intensity. Dashed lines indicate the region from which luminescence intensity was collected for d. The scale bar in (e–g) is 1 μm. (Reprinted with permission from ref. 198 and 201. Copyright 2009 and 2014, National Academy of Sciences, USA and Nature Publishing Group.)
6.3 Pulse-laser excitation

In contrast to extensive studies of steady-state excitation, less attention is paid to investigations on UC nanocrystals under excitation of pulse laser with the exception of lifetime measurements. The advantages of pulse laser excitation derive from variations in pulse in terms of repetition rate, peak power and pulse width. Owing to the power density-dependent emission of UC nanocrystals, pulse laser excitation, in principle, provides a valuable handle for spectroscopic studies.

Upon excitation with pulse-widths in the order of microsecond or millisecond comparable to the lifetimes of the intermediate states of lanthanide activators, a significant increase in quantum yield of UC nanocrystals is achievable as a result of a high fluorescence rate. For example, Liu and co-workers reported that an optimal signal gain (ratio of the quantum yield\textsubscript{pulsed}/ quantum yield\textsubscript{CW}) up to 8.7 was obtained from NaYF\textsubscript{4}:Yb/Tm (25/0.3 mol\%)@NaYF\textsubscript{4} core–shell nanocrystals under 2 Hz square-wave pulse excitation (power density: 0.12 W cm\textsuperscript{-2}; pulse width: 20 ms) (Fig. 16).\textsuperscript{203} The quantum yield of NaYF\textsubscript{4}:Yb/Tm nanocrystals doubled under excitation of a 5 KHz square-wave pulse (peak power: 1 W; pulse width: 200 \(\mu\)s).\textsuperscript{204} It is worth mentioning that the combination of high brightness of each pulse with discrete excitation facilitates realization of single-shot deep tissue imaging without autofluorescence and concerns of the overheating effect.

When the pulse width is reduced to nanoseconds which is much shorter than the lifetime of the intermediate states of lanthanide activators, it has a negligible impact on the quantum yield of the UC nanocrystals. However, for short pulse widths with high power density (10\(^8\) to 10\(^9\) W cm\textsuperscript{-2}), it is applicable to excite the nanocrystals for achieving multi-photon UC emission. Moreover, the high power density of the pulse can lead to a large population inversion for optical lasing by sequential pulse excitation of UC nanocrystal colloids using an optical parametric oscillator laser.\textsuperscript{205}

6.4 Emission characterization

Commercially available or customized luminescence spectrophotometers, coupled with an external laser source, are regarded as workhorse instruments for studying luminescent properties of UC nanocrystals (Fig. 17). A spectrophotometer section generally contains a filter, grating mirrors and a photon detector. At present, most of the photon detectors comprise a photomultiplier (PMT) for converting impinging photons into electronic charges and an optimized dynode chain for the amplification of secondary electrons. The emission spectrum of UC nanocrystals encompasses a wide range of wavelengths from UV to NIR spectral regions, thus imposing a critical requirement for PMT to effectively convert all emitting photons to electrons. The sensitivity of PMT to emissions at different wavelengths varies significantly and strongly depends on the composition of photocathodes. For example, GaAsP and GaAs photocathodes are only sensitive to the photons in the ranges of 250–650 nm and 300–800 nm, respectively. The wavelength-dependent sensitivity of PMT indicates that detector correction is necessary, especially for customized spectrophotometers prior to emission characterization. For example, a striking difference in the emission ratio of NIR-to-visible was observed from the corrected and uncorrected...
emission spectra of NaGdF$_4$:Yb/Tm (49/1 mol%)@NaGdF$_4$:Eu (15 mol%) core–shell nanocrystals (Fig. 17). The correction for emission ratio differences can be quite important when measuring quantum yields or collecting data in order to form the CIE chromaticity diagram. Moreover, owing to the multi-band emission feature of UC nanocrystals, filters in some cases are needed to prevent the generation of overtone bands. For emission measurements, a quartz vial is preferred over glass or plastic vials because they have different levels of absorption in the UV, visible or NIR region.

The choice of the host lattice and the level of impurity concentration have strong effects on the emission of UC nanocrystals measured at different temperatures. Pires et al. reported on the suppression in the emission intensity of Y$_2$O$_3$:Yb/Er UC nanocrystals with increasing temperature from 10 to 300 K, indicating the enhancement of non-radiative multiphonon relaxation at elevated temperatures.206 Yu et al. studied the effect of the crystal phase on the emission of NaYF$_4$:Yb/Er nanocrystals.207 The authors found that the emission intensity of cubic phase nanocrystals decreased monotonically as the temperature was increased from 10 to 400 K, while hexagonal phase counterparts exhibited maximum emission intensity at 100–150 K under identical experimental conditions. They ascribed this result to the competition between two optical processes: energy transfer from $^2F_{5/2}|Yb^{3+}$ to $^4I_{11/2}|Er^{3+}$ and concurrent promotion of non-radiative relaxation at elevated temperatures.208 It is interesting to note that the intensity ratio of two emissions arising from $^2H_{11/2}$ to $^4I_{15/2}$ (at 523 nm) and $^4S_{3/2}$ to $^4I_{15/2}$ (at 542 nm) transitions of Er$^{3+}$ is highly sensitive to temperature under low power density excitation. This effect can be harnessed to develop thermal sensors for use in the measurement of deep tissue temperature.209

Low-temperature luminescence spectroscopy brings a new dimension to systematic investigations of energy transfer mechanisms between the lanthanides doped in UC nanocrystals. For example, Suyver and co-workers observed that the emission intensity of NaYF$_4$:Yb/Er (18/2 mol%) nanoparticles measured at an ultralow temperature of 5 K is several orders of magnitude lower than that obtained at room temperature.19 Much of the observed phenomenon was associated with the absence of direct energy transfer from Yb$^{3+}$ to Er$^{3+}$ caused by the low temperature factor. High resolution optical measurements of UC nanocrystals at low temperature are also useful in interpreting the site symmetry of activators. On the basis of the UC luminescence spectrum obtained at 10 K for Y$_2$O$_3$:Yb/Er (1/2 mol%) nanocrystals, Güdel and co-workers predicted different site symmetries ($C_6$ and $C_{3v}$) of Y$^{3+}$ upon substitution by Er$^{3+}$ in the host lattice.206

### 6.5 Absolute quantum yield measurement

Absolute quantum yield ($\phi$) of UC nanocrystals is the direct measure of the efficiency of the conversion of NIR incident photons into visible or even UV emissions. It is defined as the ratio of the number of UC photons emitted ($N_{\text{emission}}$) to the number of photons absorbed ($N_{\text{absorption}}$) (eqn (7)).

$$\phi = \frac{N_{\text{emission}}}{N_{\text{absorption}}} = \frac{L_{\text{sample}}}{E_{\text{sample}} - E_{\text{reference}}}$$

where $L_{\text{sample}}$ denotes the integrated emission peaks, $E_{\text{reference}}$ and $E_{\text{sample}}$ are the respective integrated excitation peaks after absorption (or scattering) of the reference and specimen. One should take into account that absolute quantum yield does not correlate to the brightness of the UC nanocrystals.

The measurement of the absolute quantum yield of UC nanocrystals relies on two indispensable components: an integrating sphere by which the emission from nanocrystals is scattered and an extended spectrometer for analyzing the scattered photons (Fig. 18).210 In 1998, Page et al. measured the UC quantum yield of NaYF$_4$:Yb/Er (18/2 mol%) and NaYF$_4$:Yb/Tm (27/0.1 mol%) in bulk form through use of the integrating sphere.211 In 2010, van Veggel’s group took one step forward and first measured the absolute quantum yield of NaYF$_4$:Yb/Er (20/2 mol%) nanocrystal colloids.212 Absolute quantum yields in the range of 0.005% to 0.3% were measured for several batches of nanoparticles with particle sizes of 10 to 100 nm, while a quantum yield of 3% was obtained for a bulk control. This method has been widely utilized to determine the quantum yields of different types of UC nanocrystal colloids (Table 5). Critically, the quantum yield of UC nanocrystals without the core–shell structure is generally low ranging from 0.002 to 1.2%.78,116,180,210,212–214 By comparison, the shell-passivated UC nanocrystals showed largely improved conversion efficiencies (0.18–7.6%).180,210,212–215 Obviously, the quantum yields of nanosized UC particles is much lower than the expected theoretical value (100/n%) resulting from an $n$-photon-UC process. The low efficiency observed for UC nanocrystals is likely due to non-radiative cross-relaxation between lanthanide dopants, in addition to down-shifting emissions by sensitizers (e.g., 1030 and 1050 nm of Yb$^{3+}$) and activators (e.g., 1530 nm of Er$^{3+}$).
of the photon detector must be performed prior to the spectroscopic measurement.

6.6 Lifetime measurements

The combination of a spectrophotometer and a pulse laser is a standard instrument for investigating emission dynamics of UC nanocrystals. For example, the temporal emission evolution of the UC nanocrystals is usually recorded on a commercially available phosphorescence lifetime spectrometer coupled with a tunable mid-band optical parametric oscillator pulse laser. As illustrated in Fig. 19a and b, two types of time-resolved spectral evolution curves representing different UC mechanisms\textsuperscript{216} are commonly collected after pulse excitation: (i) single exponential decay accounting for an excited state absorption (ESA) UC process and (ii) short rise time followed by a relatively slow exponential decay, characteristic of an energy transfer UC process. The rising time in the energy transfer UC process is strongly correlated to the structure of the UC nanocrystals under investigation. For example, multilayer NaYF\textsubscript{4}:Yb/Tm (30/0.5 mol\%)@NaYF\textsubscript{4} nanocrystals require a much longer time (2.5 ms) to populate the \(^3\text{H}_4\) state of Tm\textsuperscript{3+} than other types of core–shell structured UC nanocrystals, such as NaYF\textsubscript{4}:Yb/Tm (30/0.5 mol\%)@NaYF\textsubscript{4} (1 ms) and NaYF\textsubscript{4}:Yb/Tm (30/0.5 mol\%)@NaYF\textsubscript{4} (1.3 ms).\textsuperscript{217}

The lifetime of UC nanocrystals can be drawn from the decay curve by means of different approaches. If the decay curve fits eqn (8), the lifetime is then obtained directly from the fitting. Otherwise, the effective lifetime would be used and calculated according to eqn (9).

\[
I(t) = I_0 \exp\left(\frac{-t}{\tau}\right) \quad \text{(8)}
\]

\[
\tau_{\text{eff}} = \frac{1}{I_0} \int_0^\infty I(t)dt \quad \text{(9)}
\]

where \(I_0\) and \(\tau\) are the initial emission intensity and lifetime of the analyte.

Of particular note is our inability to quantitatively compare different quantum yields reported thus far for UC nanocrystals. This is because absolute UC quantum yields depend highly on experimental conditions, especially the power density of the excitation source.\textsuperscript{211} Additionally, different brands of photon detectors may lead to considerable deviation as well. To ensure the validity of quantum yield characterization, careful correction of the photon detector must be performed prior to the spectroscopic measurement.

### Table 5: Quantum yields of typical activators doped in different nanoparticles and the corresponding experimental conditions

| Composition (mol%) | Phase     | Size (nm) | Power density (W cm\(^{-2}\)) | \(\phi\) (%) | Ref. |
|-------------------|-----------|-----------|-------------------------------|-------------|-----|
| **Bulk**          | NaYF\textsubscript{4}:Yb/Er (20/2) | Hexagonal | 20                            | 3.0 ± 0.3   | 212 |
| Core nanoparticle | NaYF\textsubscript{4}:Yb/Er (20/2) | Hexagonal | 100                           | 0.30 ± 0.10 | 212 |
|                   | NaYF\textsubscript{4}:Yb/Er (20/2) | Hexagonal | 30                            | 0.10 ± 0.05 | 212 |
|                   | NaYF\textsubscript{4}:Yb/Er (20/2) | Hexagonal | 8–10                          | 0.005 ± 0.005 | 78 |
|                   | NaYF\textsubscript{4}:Yb/Er (20/2) | Hexagonal | 5.4                           | 0.0022 ± 0.0001 | 78 |
|                   | NaGdF\textsubscript{4}:Yb/Er (20/2) | Hexagonal | 37                            | 0.14 ± 0.01 | 78 |
|                   | LiLuF\textsubscript{4}:Yb/Tm (20/0.5)@LiLuF\textsubscript{4} | Tetragonal | 28                            | 0.61       | 213 |
|                   | LiLuF\textsubscript{4}:Yb/Tm (20/0.5)@LiLuF\textsubscript{4} | Tetragonal | 28                            | 0.45       | 214 |
| Core–shell Nanocrystal | NaYF\textsubscript{4}:Yb/Tm (25/0.3)@NaYF\textsubscript{4} | Hexagonal | 38                            | 0.47 ± 0.06 | 116 |
|                   | NaYF\textsubscript{4}:Yb/Er (20/2)@NaYF\textsubscript{4} | Hexagonal | 30                            | 0.30 ± 0.10 | 212 |
|                   | NaGdF\textsubscript{4}:Yb/Er (20/2)@NaYF\textsubscript{4} | Hexagonal | 9                             | 0.19 ± 0.01 | 180 |
|                   | NaYF\textsubscript{4}:Yb/Tm (25/0.3)@NaYF\textsubscript{4} | Hexagonal | 17                            | 0.51 ± 0.01 | 210 |
|                   | NaYF\textsubscript{4}:Yb/Tm (25/0.3)@NaYF\textsubscript{4} | Hexagonal | 42                            | 3.5        | 215 |
|                   | LiLuF\textsubscript{4}:Yb/Tm (20/0.5)@LiLuF\textsubscript{4} | Tetragonal | 43                            | 1.2        | 214 |
|                   | LiLuF\textsubscript{4}:Yb/Tm (20/0.5)@LiLuF\textsubscript{4} | Tetragonal | 50                            | 3.6        | 213 |
|                   | LiLuF\textsubscript{4}:Yb/Tm (20/0.5)@LiLuF\textsubscript{4} | Tetragonal | 40                            | 6.7        | 213 |
|                   | LiLuF\textsubscript{4}:Yb/Tm (20/0.5)@LiLuF\textsubscript{4} | Tetragonal | 50                            | 7.6        | 213 |

*ex = 1490 nm.*
nanocrystals and successfully identified the location of emissive Er$^{3+}$ in the center of the core or at the core–shell interface by time-resolved spectroscopic studies.$^{219}$

Inter-ionic energy transfer is a dominating factor concerning variations in the lifetime of an emissive intermediate state of lanthanides doped in UC nanocrystals. For example, the lifetime of Gd$^{3+}$ emission at 310 nm ($^4\text{P}_{7/2} \rightarrow ^4\text{S}_{7/2}$) of NaYF$_4$:Yb/Tm (19/1 mol%)@NaYF$_4$ core–shell nanoparticles decreased significantly from 1011 to 555 μs when Tb$^{3+}$ (15 mol%) ions were incorporated into the shell layer in favor of energy transfer from Gd$^{3+}$ to Tb$^{3+}$.65,185 Similarly, the lifetime of Tm$^{3+}$ emission at 475 nm ($^3\text{F}_{4} \rightarrow ^3\text{H}_6$) of NaYF$_4$:Yb/Tm nanocrystals could be tuned from 25.6 to 662.4 μs through control over energy transfer between Yb$^{3+}$ and Tm$^{3+}$ ions by varying the molar ratio of Yb$^{3+}$-to-Tm$^{3+}$ from 20/8 to 20/0.2.$^{220}$

UC nanocrystals with tunable lifetimes hold great promise in multiplexing and document coding.$^{221}$ Emissions with different lifetimes can be spectroscopically resolved or microscopically imaged with the help of a time-gated PMT. For example, Chen and co-workers demonstrated that it is possible to separate long-lived red emission of Eu$^{3+}$ and short-lived blue emission of Tm$^{3+}$ from NaGdF$_4$:Yb/Tm (18/1 mol%)@NaGdF$_4$:Eu (10 mol%) core–shell nanocrystals by the time-resolved luminescence technique.$^{222}$ In another impressive work demonstrated by Jin and co-workers, different patterns encoded with UC nanocrystals of varied lifetimes could be interpreted in a layer-by-layer fashion through use of a time-gated microscope (Fig. 19e–f).$^{220}$ One limitation associated with such security printing application is purely technical: no known robust methods are currently available for the synthesis of lifetime-tunable UC nanocrystals displaying similar brightness.

It is worthy of note that the lifetimes of UC nanocrystals reported previously are irrespective of the excitation power density up to 500 W cm$^{-2}$.223 However, a recent study has confirmed that upon excitation with a high power density (e.g., 10$^6$ W cm$^{-2}$), the lifetimes of the intermediate states responsible for visible emission photons would be shortened as a result of elevated energy transfer rates and cross-relaxation processes.$^{201}$

6.7 Energy transfer measurement

The characterization of energy transfer between UC nanocrystals and other suitable energy acceptors or occasionally energy donors, including organic dyes, quantum dots and semiconducting thin films is of fundamental importance to designing high-sensitivity biosensors and improving NIR light harvesting of photovoltaic cells.$^{27,224}$ Non-radiative energy transfer (Dexter exchange or the Förster resonance mechanism) and radiative energy transfer are the two major pathways that govern the energy transfer of UC nanoparticle-involved systems.$^{229,230}$ The efficiency of energy transfer ($E$) between the energy donor and acceptor can be quantified by the fraction of an energy transfer event occurring per donor excitation event (eqn (10) or (11)):

$$ E = 1 - \frac{I_{DA}}{I_D} \quad (10) $$

or

$$ E = 1 - \frac{\tau_{DA}}{\tau_D} \quad (11) $$

where $\tau_D$ and $\tau_{DA}$ are the respective luminescence lifetime of the donor in the presence and absence of the acceptor, while $I_D$ and
$I_{DA}$ are the respective integrated intensity of the energy donor measured with and without the acceptor.

Sharp emission bands of lanthanide activators make UC nanocrystals suitable as excellent energy donors, allowing one to construct energy transfer systems involving a wide range of energy acceptors, including plasmonic nanocrystals, organic dyes, quantum dots and metal oxides.\textsuperscript{120,225-227}\hfill 

In such systems, the efficiency of energy transfer is within the range of 10–90%, depending on the relative molar ratio between the energy donor and energy acceptor. For example, Wang et al.\textsuperscript{208} reported that the green emission of NaYF$_4$:Yb/Er (4/2 mol%) nanocrystals at 543 nm could be effectively transferred to gold nanoparticles with a high efficiency up to 70%.\textsuperscript{227} To optimize the energy transfer efficiency, an inert-shell with critical thickness of several nanometers is essential for preserving energy of excited donors, while inducing negligible separation between the energy donor and acceptor.\textsuperscript{67}

Non-radiative energy transfer at a short range typically renders a higher efficiency relative to the energy transfer via a radiative pathway. As a result, it is deemed that the radiative energy transfer plays a trivial role in the energy transfer process.\textsuperscript{61,67,228,229} For example, Riuttamäki and colleagues confirmed a non-radiative energy transfer from UC nanocrystals to fluorescent dyes in aqueous suspensions, as evident by a decreased lifetime of UC nanocrystals, without concerning radiative energy transfer.\textsuperscript{230}

To achieve broadband excitable UC nanocrystals, recent studies have shifted the focus of research on developing UC nanocrystals for use as energy acceptors. For example, a relatively efficient energy transfer ($E \sim 50\%$) from organic dyes to NaYF$_4$:Yb/Er (20/2 mol%) nanoparticles was observed, generating a new class of UC nanomaterials excitable by a broadband from 740 to 850 nm.\textsuperscript{231} Despite the success, two concerns were presented in their study: (i) low UC efficiency of the dye-sensitized UC nanocrystals due to back-energy transfer to the dye molecules and down-shifting emission of the activators and (ii) low photostability of the hybrid system arising from the photobleaching effect of the dye molecules. The second concern was confirmed by Hyppänen et al., who illustrated that almost 50% of IR-806 dye molecules were bleached after 30 s of laser beam irradiation (power density: 9.5 W cm$^{-2}$).\textsuperscript{232}

A Förster resonance energy transfer model, suitable for the situation between molecular donors and acceptors, has been utilized to study the efficiency of energy transfer between UC nanocrystals and suitable energy acceptors in close proximity (eqn (12)).

$$E = \frac{1}{1 + R^6/R_0^6} \tag{12}$$

where $R$ refers to the distance between the donor and acceptor; $R_0$ (known as critical distance) is the distance at which the efficiency of energy transfer is 50% and is typically within the range of 1–10 nm in most cases.\textsuperscript{233} It should be noted that as the size of UC nanocrystals (20–30 nm) is much larger than the critical distance, eqn (12) is in theory unable to model the energy transfer between the UC nanocrystals and other species.\textsuperscript{230} Moreover, it is especially difficult to determine $R$ for UC nanoparticle-mediated energy transfer systems due to the fact that the energy donors (lanthanide activators) are randomly distributed in the particle.

Energy transfer between different lanthanide activators, doped in two neighboring segments of a microstructure, can be probed by measuring the lifetime of the activators. For example, Zhang et al.\textsuperscript{121} reported the synthesis of a dual-color emitting microrod by incorporating Tm$^{3+}$ and Er$^{3+}$ activators into the central segment and two tips of the microrod, respectively (Fig. 20).\textsuperscript{211} Using a confocal microscope equipped with a lifetime spectrometer, the researchers found that the green ($^4S_{3/2} \rightarrow ^4I_{15/2}$) and red ($^4F_{9/2} \rightarrow ^4I_{15/2}$) emission lifetimes of Er$^{3+}$ measured from different parts of the microrod are essentially the same, implying that no obvious interfacial crosstalk between Tm$^{3+}$ and Er$^{3+}$ occurs. Critically, this technique has limitations for measuring energy transfer between microdomains within a microsized particle because of relatively large size in the focal spot (400 nm in radius) of the confocal microscope. Combined with the recently emerged stimulated emission depletion (STED) method, this approach may enable us to precisely map emission profiles within a single UC nanocrystal because of the high resolution capability of STED ($\sim 2.4$ nm).\textsuperscript{234}

6.8 AFM-coupled spectrometer for single particle imaging

An AFM-coupled spectrophotometer is an integrated instrumental setup capable of measuring the size, morphology, and optical properties of single UC nanocrystals simultaneously (Fig. 21a).\textsuperscript{235} The combination of AFM with optical spectroscopy can largely
simplify the characterization process for single UC nanocrystals as compared to conventional light microscopes.

With the help of AFM, Schietinger and colleagues reported an abnormal size-dependence of the UC emission, on the single-particle level, for NaYF₄:Yb/Er nanocrystals.²³⁶–²³⁸ In their report, the authors found that the emission intensity ratio of green-to-red measured from single nanocrystals can be quite different, depending on their size. They attributed this aberration to different degrees of phonon confinement in nanocrystals of dissimilar sizes. The authors further utilized their instrument setup to elucidate plasmonic effects on UC emission by \textit{in situ} monitoring the variations in the emission intensity of a UC nanoparticle in response to changes in the distance between the UC nanoparticle and a gold nanoparticle, placed in close proximity (Fig. 21b).²³⁹ \textit{In situ} optical characterization revealed that apart from an enhancement in the UC emission of the nanoparticle with an overall factor of 4.8 and 2.7, respectively. (Reprinted with permission from ref. 236 and 239. Copyright 2009, American Chemical Society.)

6.9 \textit{In vitro} and \textit{in vivo} imaging measurement

With UC nanocrystals designed as luminescent biomarkers to target cells and tissues, optical microscopes can be exploited for investigating cellular and tissue functions \textit{in situ} or \textit{in vitro}.²⁴¹–²⁴³ For example, a conventional wide-field fluorescence microscope coupled with a CCD camera was applied to acquire high quality cell images in the presence of UC nanocrystals, benefiting from the absence of autofluorescence.²⁴⁴,²⁴⁵ To further improve subtle depth discrimination and achieve 3D visualization of the specimen under investigation, confocal microscopy can be furnished by virtue of its point-by-point scanning capability, equipped with a pinhole aperture to block the signals generated by the sample outside of the optical focus. By modifying a common inverted microscope, Li and co-workers constructed a laser scanning confocal UC luminescence microscope having a spatial resolution comparable to that of a conventional confocal microscope (Fig. 22a).²⁴⁶ As the imaging is based on the detection

Fig. 21 Characterization of UC nanocrystals on the single-particle level through use of an AFM-coupled spectrophotometer. (a) Schematic illustration of the instrument setup. (b) Luminescence emission spectra of a single UC nanocrystal measured in the presence (blue curve) and absence (violet curve) of a gold nanoparticle (see the inserted panels) in close proximity. Note that the green and red emissions of the nanocrystal are enhanced by a factor of 4.8 and 2.7, respectively. (Reprinted with permission from ref. 236 and 239. Copyright 2009, American Chemical Society.)

Fig. 22 Schematic setup used for \textit{in vitro} and \textit{in vivo} imaging of cells and animals. (a) Schematic illustration of a laser scanning UC luminescence confocal microscope for \textit{in vitro} study. (b) Experimental setup for \textit{in vivo} imaging of small animals using UC nanocrystals as biomarkers. (Reprinted with permission from ref. 173 and 246. Copyright 2009, American Chemical Society.)
of anti-Stokes luminescence from UC nanocrystals, a short-pass excitation dichroic mirror is required to separate the emission from NIR excitation. Many other groups also demonstrated the benefit of using the scanning confocal UC luminescence imaging for high-resolution cell imaging and particle trafficking within the cells.\textsuperscript{35,116,158,198,247-251}

For small animal non-invasive imaging involving UC nanocrystals, a variety of imaging modalities can be developed, including tumor-targeted imaging, lymphatic imaging, vascular imaging, and cell tracking. For example, a representative in vivo imaging system was constructed by combining a 980 nm laser with a high quality CCD camera and a filter to eliminate scattered NIR light.\textsuperscript{85,242,252} Note that commercially available in vivo imaging systems for small animals are difficult to apply to UC luminescence imaging because high-quality short-pass filters are required to attenuate the strong interference from the excitation source.\textsuperscript{20,173,241} A more detailed discussion of the customized instrumental setup for small animal imaging was presented by Xiong et al. (Fig. 22b).\textsuperscript{173} In a typical imaging experiment, two external adjustable CW 980 nm lasers were adopted as an excitation source, and an Andor DU897 EMCCD was utilized as a signal collector. A filter was placed in the front of the collector to block the scattered excitation photons. Taking advantage of this setup, short-term (24 h) and long term (115 days) in situ tracking of the distribution of NaYF\textsubscript{4}:Yb/Er/Tm nanocrystals in mice were conducted (Fig. 23).\textsuperscript{19,173} The results showed that a large portion of the nanocrystals was first accumulated in the liver and spleen, and further excreted from the body by hepatobiliary transport. Notably, the results on the retention time from long tracking (up to 90 days in intestinal tract) is slightly different from those obtained from ICP-AES measurement (7 days) reported by Zhang’s group.\textsuperscript{125} Through use of β-NaLuF\textsubscript{4}:Gd/Yb/Tm nanoparticles, Li and co-workers achieved a detection limit of less than 50 cells for whole-body luminescence imaging of a small mouse with a penetration depth of \~2 cm.\textsuperscript{116} A major challenge of this in vivo UC imaging approach is the development of the high throughput systems to evaluate a large number of small animals for screening purposes.

7. Evaluation of magnetic properties

Lanthanide-doped UC nanocrystals not only have excellent optical characteristics but also possess attractive magnetic properties which may hold promise as bioprobes for multimodal imaging.\textsuperscript{174,196,253} The magnetic properties of UC nanocrystals mainly derive from lanthanide ions with unpaired inner 4f electrons, such as Gd\textsuperscript{3+}, Nd\textsuperscript{3+}, Dy\textsuperscript{3+} and Ho\textsuperscript{3+}. Owing to the shielding effect of 5s- and 5p-electrons, these ions have localized, noninteracting magnet moments, giving rise to paramagnetism.\textsuperscript{254} The magnetic properties of the nanocrystals are normally investigated by a commercially available SQUID magnetometer or VSM. The paramagnetic behavior observed at room temperature for GdF\textsubscript{3} and NaGdF\textsubscript{4} nanocrystals is drastically different from that of Gd metal (ferromagnetism) due to poor overlapping of 4f orbitals of neighboring Gd\textsuperscript{3+} ions in host materials (Fig. 24).\textsuperscript{255,256} The magnetization and magnetic mass susceptibility of UC nanocrystals are sensitive to the content of magnetic lanthanide ions in host matrices. For instance, the magnetic mass susceptibility of pure NaGdF\textsubscript{4} nanocrystals (16 nm) was changed from 9.29 \times 10^{-5} to 7.46 \times 10^{-5} emu g\textsuperscript{-1} after co-doping of Yb\textsuperscript{3+}/Er\textsuperscript{3+} (20/0.2 mol%).\textsuperscript{257} Alternatively, the magnetic properties of the nanocrystals can be manipulated by introducing non-magnetic lanthanide ions such as Y\textsuperscript{3+}.\textsuperscript{256,258}

![Fig. 23](image_url)  
**Fig. 23** Time-dependent in vivo UC luminescence imaging of subcutaneous U87MG tumor (left hind leg, indicated by short arrows) and MCF-7 tumor (right hind leg, indicated by long arrows) borne by athymic nude mice after intravenous injection of RGD-coupled UC nanocrystals over a period of 24 h. (a–c) 1 h. (d–f) 4 h. (g–i) 24 h. Note that columns 1, 2 and 3 are respective bright-field image, UC luminescence image and overlay of luminescence and bright-field images. (Reprinted with permission from ref. 173. Copyright 2009, American Chemical Society.)

![Fig. 24](image_url)  
**Fig. 24** Magnetization of NaGdF\textsubscript{4}:Yb/Er (20/0.2 mol%) and NaGdF\textsubscript{4} nanocrystals as a function of the applied field. Note that inset shows luminescent photographs of NaGdF\textsubscript{4}:Yb/Er nanocrystals, recorded under excitation at 980 nm, with and without a magnet, indicating that these UC nanocrystals can be recovered by applying an external magnetic force. (Reprinted with permission from ref. 256. Copyright 2011, American Chemical Society.)
transverse (T2) relaxation time of water protons. A mass effect on relaxation times are more suitable as negative contrast agents for shortening the relaxation time.

Small-sized NaGdF4 or GdF3 nanoparticles with a high doping content of Gd3+ are preferred in order to achieve a better T1 enhancement because of its concentration dependence of Gd3+ ions in close proximity to water molecules. Alternatively, a thin NaGdF4 shell coating of lanthanide-doped UC nanoparticles can render the nanoparticles with both improved T1 performance and enhanced UC emission. By comparison, Dy3+-containing nanocrystals are more suitable as negative contrast agents for shortening the transverse (T2) relaxation time of water protons. A mass effect on T2 relaxation has been reported by van Veggel’s group using NaDyF4 UC nanoparticles. Their study suggested the use of large-sized Dy3+-based nanocrystals to enable high spatial and temporal resolution for T2 performance.

### 8. Conclusion and perspective

Over the past decade, remarkable progress has been achieved in the field of UC nanocrystals. Many of these nanocrystals combine novel optical properties with simple preparation techniques and offer the scope of tuning the material’s structure or composition to give desired emission profiles, making them attractive for use in a broad range of applications. Along with the advances in the fabrication of UC nanomaterials, there also has been a corresponding increase in the development of new analytical techniques and characterization tools made possible by recent breakthroughs in nano- and micro-technology. Despite the increase in the number of techniques, existing characterization methods, however, are not meeting the needs of those concerned with the ultimate performance of UC nanocrystals. The lack of this ability has not been fully appreciated within the UC community. The reason for this lack of ability is that the characterization of UC nanocrystals is inherently more difficult than that of other luminescent nanomaterials, arising largely from the material and optical complexity associated with lanthanide dopants. Up to this point in time, no research group has been able to report the exact concentration of a specific lanthanide in a UC nanoparticle, which can be partly ascribed to the low doping concentration beyond the limits of currently available instruments.

Another practical challenge on the path toward the complete characterization of UC nanocrystals is not so much the availability of characterization techniques, but related to the question of how to combine multiple existing techniques to address the situation at hand. For a particular UC system, the success of this integrative approach requires in-depth knowledge of lanthanide chemistry as well as the extraordinary ability to extend the techniques of basic characterization to the more complicated cases, while knowing both their limitations and applicability. As research continues to progress in this area, we would not be surprised if major breakthroughs in UC nanocrystals are forthcoming.

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