

Synthesis of Water Well-dispersed PEGylated Iron Oxide Nanoparticles for MR/Optical Lymph Node Imaging

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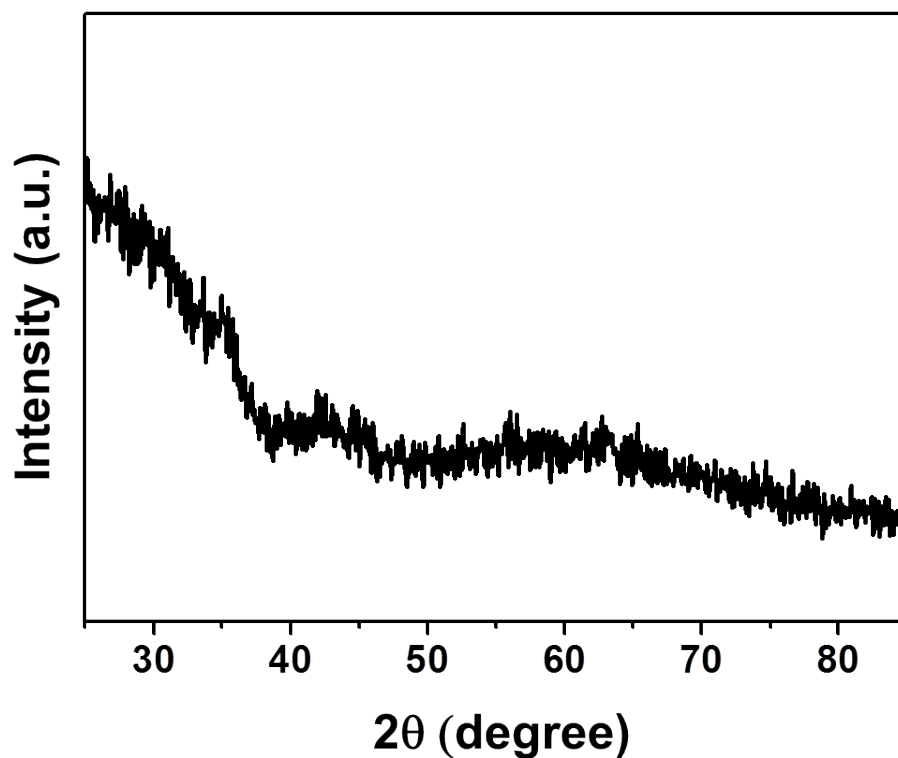


Fig S1 XRD pattern of the iron oxide nanoparticles prepared at 210°C

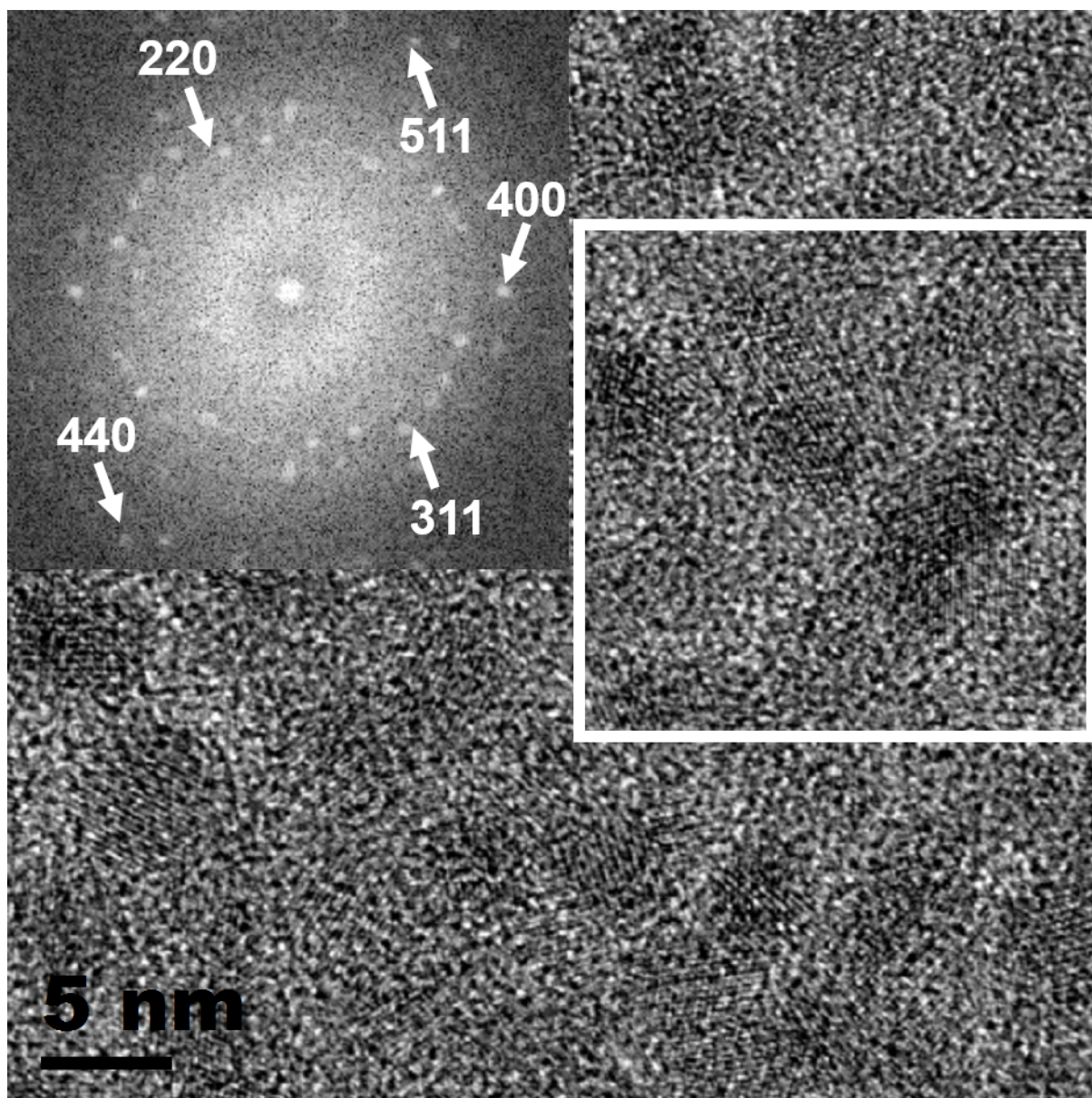


Fig S2 HRTEM image of the USPIO. Inset is a FFT pattern of the selected region.

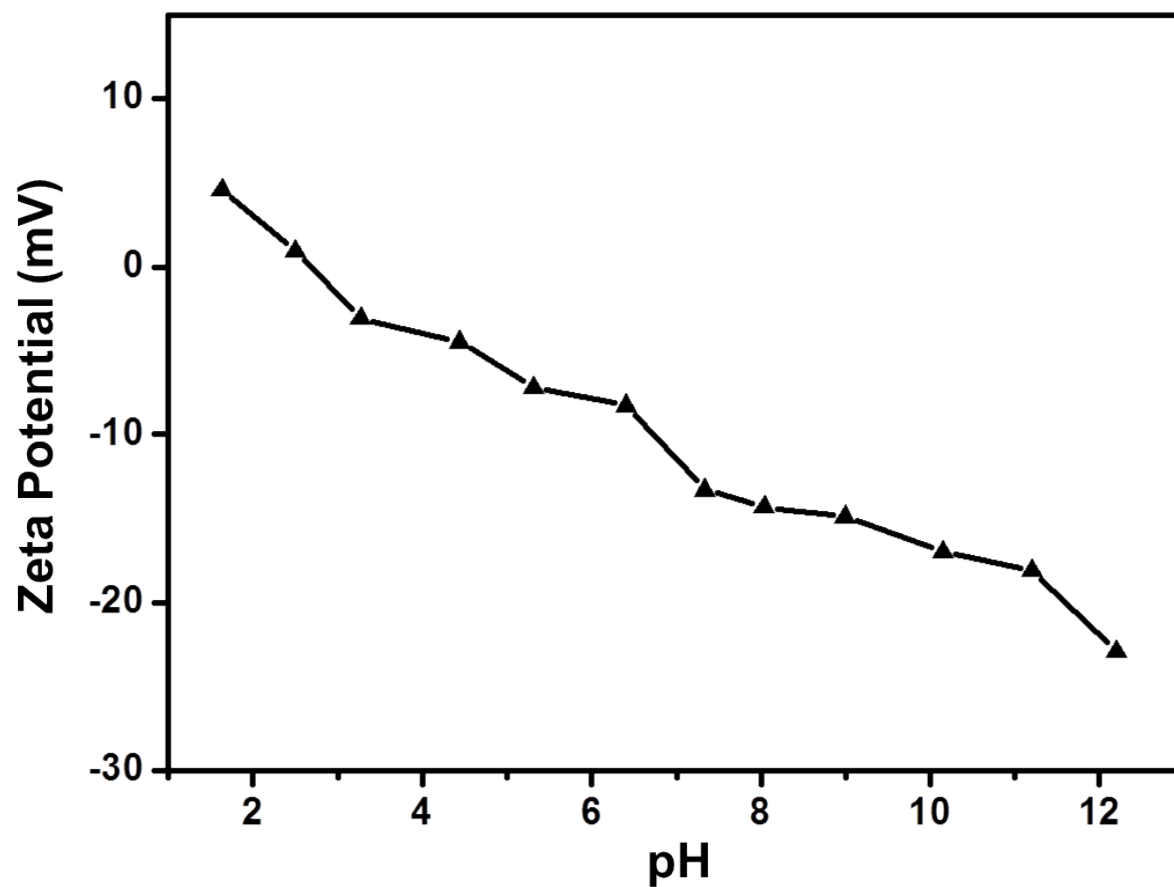


Fig S3 Zeta potential of the USPIO in various pH conditions.

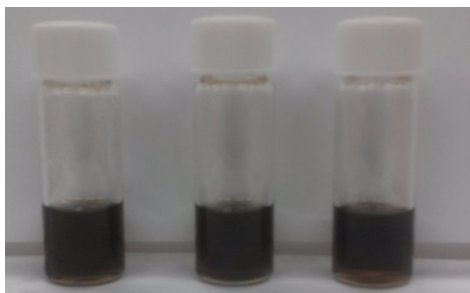


Fig S4 Photograph of the dispersion of USPIO in various solvents (DMF, DMSO, Chloroform; from left to right.)

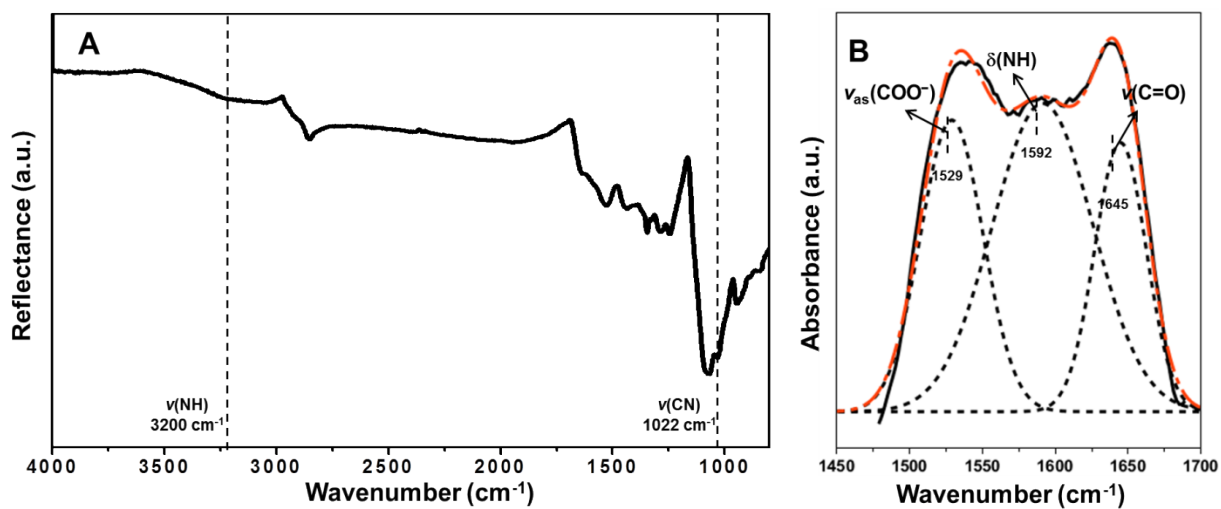


Fig S5 FT-IR spectra of the amine-functionalized USPIO (A, B) and FT-IR spectra of the Gaussian fitted amine-functionalized USPIO in 1450 - 1700 cm⁻¹ ranges

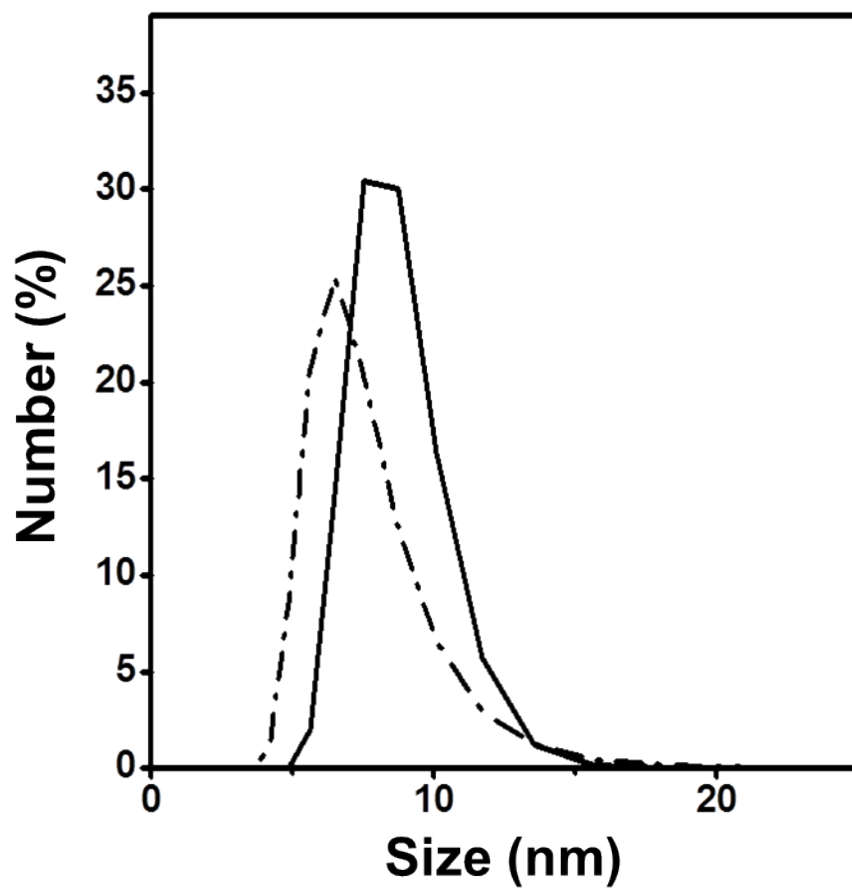


Fig S6 Hydrodynamic sizes of the USPIO (Dashed dot line) and FITC@USPIO (Solid line)

Nanoparticle quantification

The basic assumption:

All of the iron oxide nanoparticles are considered to be a spherical shape and then applied to the size distributions of particle, which were observed by TEM images as shown in Fig 1C

The magnetite unit cell parameters are $a=b=c=8.39\text{\AA}$, which translates to a unit cell volume of 590.5\AA^3 , which have 8 Fe_3O_4 units per unit cell. Total number of Fe & O atoms per unit cell is 24 and 32 respectively. The weight of a one unit cell can be calculated from the weight of individual Fe atoms ($55.845\text{ g} / 6.02 \times 10^{23} = 9.28 \times 10^{-23}\text{g}$) and O atoms ($16\text{ g} / 6.02 \times 10^{23} = 2.66 \times 10^{-23}\text{g}$) to be a $307.6 \times 10^{-23}\text{g}$.

The volume of each nanoparticle was calculated from the ideal spherical shapes = $4/3\pi r^3$

Number of unit cell per nanoparticle = Volume of the one nanoparticle divided by unit cell volume

The weight of a one nanoparticle = Weight of the unit cell \times Number of unit cell per nanoparticle + $41.5 / 58.5 \times$ Weight of magnetite portion of USPIO (The weight of PEG molecules on the surface of one nanoparticle was calculated based on the TGA result)

PEG molecule density on the surface of USPIO = (Mole of the PEG conjugated onto the nanoparticle surface \times Avogadro constant) / $4\pi r^2$

Calculations of nanoparticles are summarized as below in table S1

Volume of one nanoparticle	Amount of unit cell per nanoparticle	Weight of a one nanoparticle	Amount of PEG molecules conjugated onto the surface of each USPIO	PEG molecule density on the surface of USPIO
($\times 10^{-26}\text{ m}^3$)	(ea.)	($\times 10^{-20}\text{ g}$)	($\times 10^{-23}\text{ mol}$)	(ea. / nm^2)
3.9 ± 1.0	65.7 ± 17.0	34.5 ± 8.94	23.9 ± 6.9	2.6 ± 0.2

Table S1 The summarized table of quantifications of nanoparticles

Concentration of standardized NaOH solution: 0.1 N				
Concentration of HCl solution for back-titration: 0.1 N				
Volume of NaOH solution for back-titration: 10.00 mL				
Amount of used NaOH: 1 mmol				
Volume of HCl solution (mL)	Amount of HCl (mmol)	Amount of NaOH reacted with surface acid sites (mmol)	Amount of total PEG molecules conjugated onto USPIO / 100 mg (mmol)	Percentage of free carboxylic acid group
9.87	0.987	0.013	0.069	18.7 %

Table S2 Base-acid back titration results of USPIO

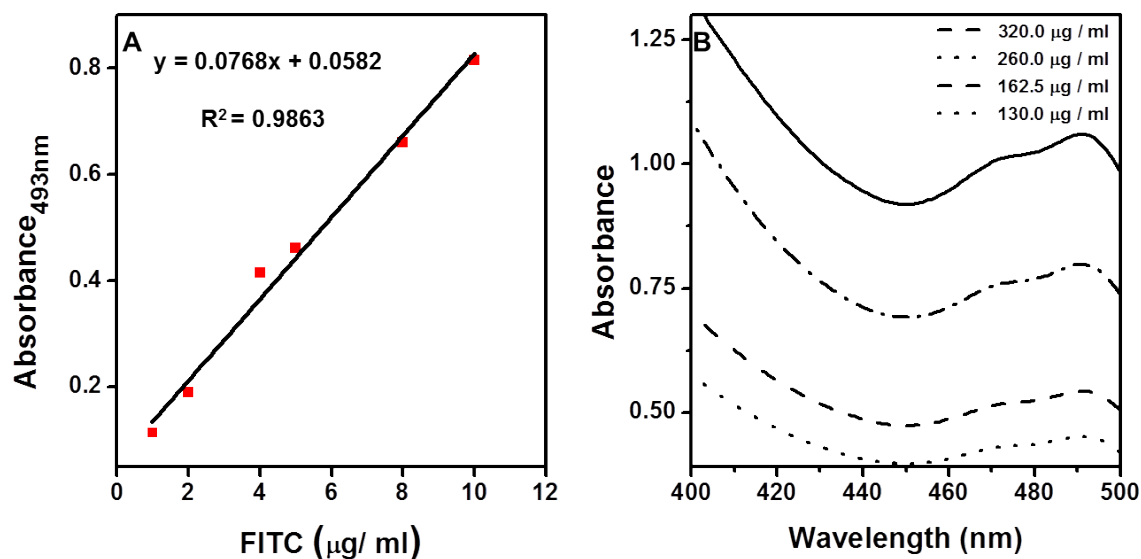


Fig S7 FITC standard curve recorded as a function of dye concentration and measuring the absorbance at 493nm (A). FITC@USPIO absorbance curve recorded as a function of USPIO weight (B). The FITC and FITC@USPIO were dispersed in distilled water.

Concentration of USPIO (ug / ml)	Absorbance	Concentration of FITC conjugated onto the surface of USPIO (umol / ml)	Amount of USPIO ($\times 10^{14}$ ea. / ml)	FITC molecules conjugated onto each USPIO
130.0	0.45	0.013	3.76	20.94
162.5	0.54	0.016	4.70	20.68
260.0	0.79	0.024	7.53	19.72
325.0	1.057	0.033	9.41	21.37
				20.7 ± 0.70

Table S3 Quantification of FITC molecules conjugated onto each USPIO. Amount of USPIO (ea. / ml) was calculated based on the Fe concentration of USPIO measured by ICP with basic assumption.

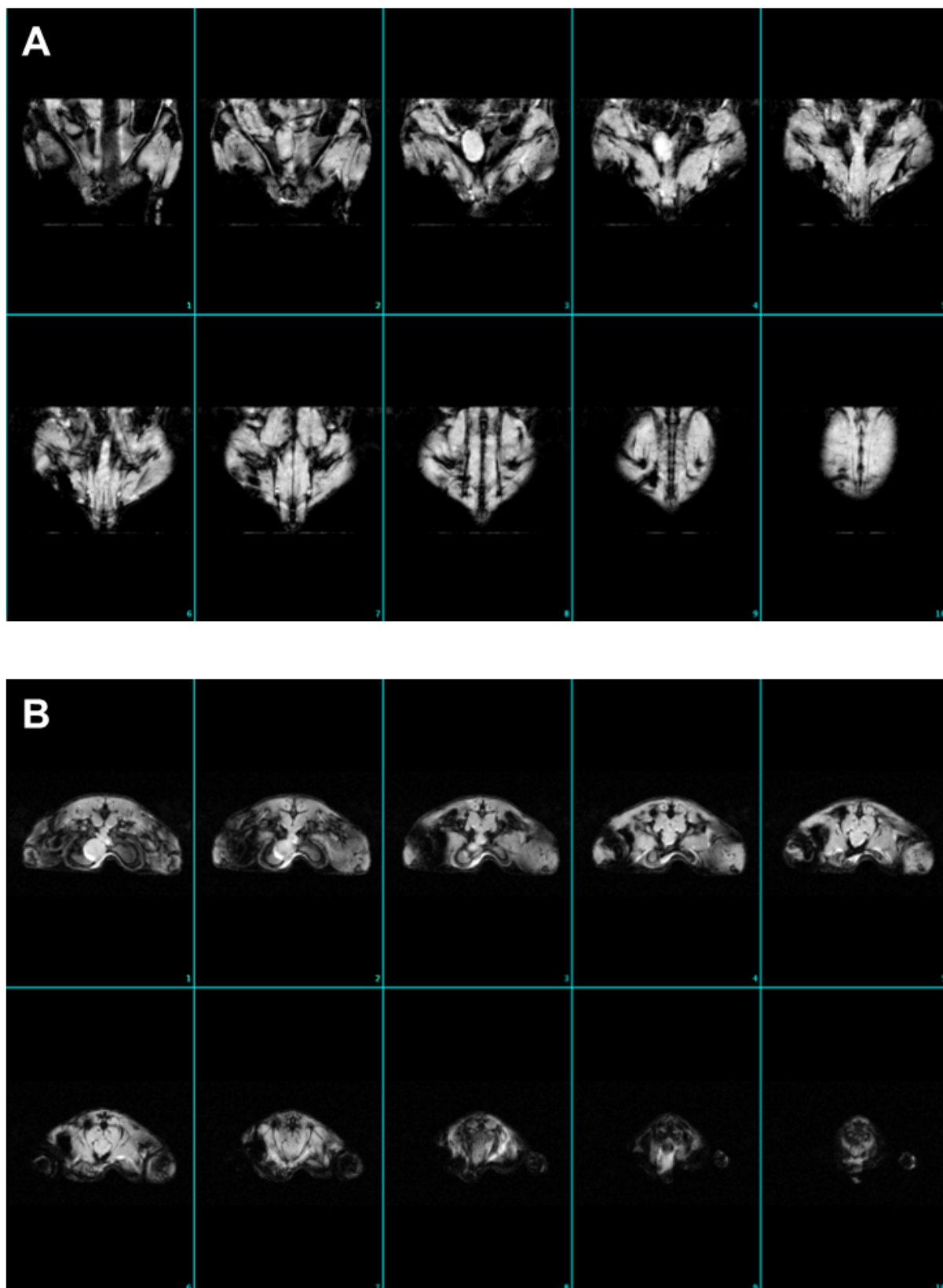


Fig S8 A series of *In vivo* T2* coronal MR images after injection of FITC@USPIO (A) for 30 minute, A series of *In vivo* T2* axial MR images of lymph node after injection of FITC@USPIO for 30 minute.