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Internal temperature sensing in lentil root seedlings with Yb, Er: $NaYF_4$ up-converting nanoparticles assisted with smartphone by using an RGB luminescent nanothermometric model

MASTER'S DEGREE FINAL PROJECT

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Internal temperature sensing in lentil root seedlings with Er,Yb: $NaYF_4$ up-converting nanoparticles assisted with smartphone by using an RGB luminescent nanothermometric model

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ABSTRACT:

Lentil root plants were grown in a controlled environment and watered with a colloidal dispersion of 0.5 µg/mL Er,Yb:$NaYF_4$ nanoparticles in water. The Er^{3+} up-conversion luminescence emission bands from this type of nanoparticles at 520 nm ($^2H_{11/2} \rightarrow {}^4I_{15/2}$), 545 nm ($^4S_{3/2} \rightarrow {}^4I_{15/2}$) and 650 nm ($^4F_{9/2} \rightarrow {}^4I_{15/2}$) after excitation of Yb^{3+} at 980 nm and the subsequent energy transfer to Er^{3+}, show good properties for luminescence nanothermometry purposes to sense temperature values in the internal parts of a lentil root plant from outside. The objective of using this luminescent nanothermometer as a tool in the field of crop temperature control, requires the simplification of experimental set-ups. For this reason here is proposed to use smartphone cameras as detectors with the application of an RGB approach for analytical treatment. The results obtained show the ability of Er,Yb:$NaYF_4$ up-conversion nanoparticles to be used as luminescent nanothermometers for temperature sensing inside the lentil plant root structure, which indicates the statement that plants have a regulative thermal mechanism against extreme temperatures, and presents the smartphone camera as a detector for the emitted luminescence.

1. INTRODUCTION

Over the past years, the approach in many fields of science has been to introduce the usage of new materials at the nanoscale in their investigations due to properties that only appear at that size scale such as quantum confinement or quantum entanglement. In this paper, the aim is to apply these nanomaterials into the plant field of study, due to a recent approach where the use of nanoparticles (NPs)[1] have shown an improvement of the plant growth and development [2,22], by increasing the photosynthetic production rate in pumpkin seedlings, resulting in growth enhancement[22]. This seems to be related to the properties of these nanoparticles (Er,Yb:$NaYF_4$)[3]. Further investigation was done regarding absorption or possible drawbacks for these NPs, demonstrating that they can indeed be absorbed by the plant biological system, and that at specific concentration rates, no harm is produced to the plant [4]. For this reason, this type of luminescent nanoparticles has shown to be a promising and helpful tool in the field of crop growth and development.

These NPs are able to generate up-conversion (UC) luminescence emissions[6]. This process happens following the mechanism of absorbing sequentially two low energy photons and its conversion to the emission of a higher energy photon. In lanthanide doped $NaYF_4$ NPs, the up-conversion process can happen on transitions among the electronic levels of the lanthanide (Ln^{3+}) ions[6], so that they can work as sensitizer (energy receivers) and as activators (emitters of the energy). $NaYF_4$ has been demonstrated to be a proper crystalline host which allows different lanthanide elements to be placed inside its structure while obtaining a high luminescence yield [5]. The lanthanide ions that are chosen to be the sensitizer and emitter, are Yb^{3+} and Er^{3+}, respectively, since this pair of Ln^{3+} are the optimal to achieve good luminescence emission in the green part of the visible spectra when excited with near-infrared (NIR) sources[7]. In fact, the main benefits of these UCNPs are related to the absorption in the NIR, which solves problems related to biological damage that could be produced when irradiating a luminescent material with ultraviolet (UV) light[7], while at the same time lifetime of the UCNPs is elongated [5].

Outside of the crop enhancement or related fields of study, these luminescence nanoparticles may be used for luminescence nanothermometry [8]. Through it, high spatial, non-invasive and remote thermometers at the nanoscale can be developed, [9] according to how the temperature of the environment surrounding the NPs affect to their luminescence characteristics, so that temperature sensing can be done by analyzing the light emitted [9]. Luminescence thermometry has been used to determine temperature in *ex-vivo*, *in-vivo* and *in-vitro*[10] applications. Different materials can be used as luminescent nanothermometers, including quantum dots[11], organic dyes[12], and Ln^{3+} doped materials[13], among others. This last type of materials attracted more interest due to their NIR range excitation[7], over the others.

Despite the effect of UCNPs on plants development has been shown to be really promising[1], their use to measure the internal temperature of the plants has not been investigated up to now. This might be of big interest regarding the biological properties of the plants and promote their optimum development. The premise is that plants can thermally regulate their internal temperature depending on the outer media [14], but no one has been able to measure that temperature and probe this fact. This can affect mainly crops production, and especially when they are cultivated in greenhouses, because the growth and development rates of a plant can radically differ even with low variations of temperature [15]. Given that the only non-invasive way of measuring the temperature of a plant is by environment sensing, that has proven to be not the most optimal way. For that reason, we propose to use Er,Yb:$NaYF_4$ NPs as a bio-compatible luminescent nanothermometer[2] for internal temperature sensing of plants which could be a useful tool for crop production improvement, as well as to prove the statement about temperature regulation in plants.

The experimental method for determining temperature by measuring light intensities is done by using proper and expensive light detectors. This is a viable solution in a laboratory environment but not in a crop field where the application aims to be used, due to cost of the set-up and the lack of robustness of these equipment and the alignment requirements to perform the measurements. For that reason, here an approach to transform this set-up into an affordable and easy to use one, by using a smartphone camera as the luminescent detector. An actual smartphone camera sensor is in fact a RGB detector [16]. Thus, if emissions are produced in two of the red-green-blue regions of the emission spectra, their intensity ratio can be used for luminescent thermometry purposes, as is the case of Er,Yb:$NaYF_4$, with emissions in the green and in the red. So, the smartphone camera sensor offers the ability to filter between colors but also they absorb all light photons resulting in an increase of the light sensitivity [17].

Here a new application for Er,Yb:$NaYF_4$ UCNPs for internal temperature sensing in plants is presented. And due to time limitations an approach of what will the smartphone as an assisting tool.

2. EXPERIMENTAL METHODS

2.1 Physical parameters of Er,Yb:$NaYF_4$ nanoparticles

The up-conversion nanoparticles used for this project were commercially acquired from Boston Applied Technologies, Inc. [18]. The lanthanide elements present in the $NaYF_4$ crystalline host were Erbium and Ytterbium. The crystalline structure of Er,Yb:$NaYF_4$ NPs is hexagonal with space group $P6_3/m$ as can be seen in the JCPDS 00-016-0334 reference pattern [19].

X-ray diffraction data and Transmission electron microscopy images of the particles can be found in figure 1 of supplementary information.

2.2 Growth of lentil seedlings

The lentil seeds were placed between two cotton layers, both layers were previously watered with 5 mL of tap water. The pH of the tap water used was always analyzed with an electronic pH meter (Pancellent TDS PH) to assure to be fulfilling the requirement for the lentil plants of neutral pH= 7[22]. The following days, the plant samples were watered with a total amount of 15 mL of water with the help of a syringe. The seedlings were completely formed after 10 to 14 days, as could be seen by the appearance of a first set of roots in the bottom layers of cotton. After that, the amount of water used was increased up to 30 mL, to ensure that it kept growing without producing any putrefaction in the under layers or cotton due to a water excess, until the plant specimen died. The process is resumed in Table 1 of the supporting information.

The watering of the plants with a colloidal dispersion starts at the 14th day when as mentioned the seedlings are formed. The colloidal dispersion of Er,Yb:$NaYF_4$ NPs has a concentration of 0.05 µg/mL. Previous publications[22] demonstrated that this concentration didn't produce any adverse effect on the plant specimen.

2.3 Environmental scanning electron microscopy (ESEM)

The morphological analysis of the root samples was done by ESEM. This electron microscopy technique was used to discriminate the presence of NPs inside or on the surface of the lentil roots. Surface observation of the seedlings was performed by placing them under low vacuum and using an acceleration voltage in the electron beam up to 15kV to avoid the saturation of the detector. To confirm the presence of the NPs in the internal parts of the roots, micro elemental analysis were performed in order to state the presence of elements related to Er,Yb:$NaYF_4$ NPs in selected points of the roots.

2.4 Thermometric calibration of Er,Yb:$NaYF_4$ NPs

For measuring the variation of the luminescence of the Er,Yb:$NaYF_4$ NPs with temperature a handmade capsule sample holder, composed of sample holder, two metallic rings and an optical microscope coverslip, was used to maintain constant the temperature in the reduced space were the nanoparticles are contained. The capsule holder was placed in a microlum set-up, under a microscope objective, with a magnification of 20x. The excitation source was a diode laser with fixed emission at 980 nm, with a beam diameter of 3000 µm on the sample. A power of 3.005 W was used in the experiments.

The emitted luminescence was collected with the same microscope objective and directed to a short-pass dichroic filter (Edmund Optics) which will eliminate all wavelengths over 750 nm, including the excitation source and any emission from the samples in the NIR. Finally, the emission was recorded using an Ocean

Flame spectrometer, and graphically represented with the respective software (Ocean View).

The variation of temperature in the NPs was produced by placing the handmade capsule over a water container, previously frozen for temperatures under room temperature that was let defrost naturally until reaching temperature. The temperatures at which luminescence spectra were recorded were controlled through a thermocouple (Eurotherm 2408i). For temperature above room temperature, the handmade capsule was placed over a laboratory heating plate (Ika RCT Basic S000), and again the temperatures at which the luminescence spectra were collected were controlled with the thermocouple.

2.4.1 Temperature plant measurements

The measurements of the luminescence under different temperatures for the NPs that were inside of the lentil plant roots was held following the same procedure as before. Modifications in the set-up, in order to facilitate the excitation of the plants, were done.

The samples were placed in the experimental set-up briefly after removing them from their seeding place. To induce a thermal stress in the lentil plants, they were placed 1h inside a refrigerator, at a temperature around 12ºC, and then their luminescence was measured. With the same purpose, but for temperatures over room temperature, the sample plants were placed near a heating furnace where the temperature was around 35ºC. The experiment was repeated over a 3-day span, to check the reproducibility. The control temperature sensors for measuring the external temperature of the lentil root environment were an Infrared thermometer (Raytek® Raynger ST™) and the thermocouple.

2.5 Use of the smartphone camera as the thermometric sensor

The intensity of the luminescence data were recorded using the external cameras of a Xiaomi Redmi Note 4 and Xiaomi Mi Note 10 Lite smartphones. Two different smartphone models were used to collect data samples with different cameras sensors with the aim of producing a model which will be independent of the pixel resolution from a specific smartphone (producing limitations regarding the ratio of pixels to the total data (pixel-ratio)). The visual data files obtained were treated with MATLAB Software[24], in which an application based on the RGB model[25] split the original data file into three different files, each one for each color channel (Red, Greed and Blue). After, a mathematic treatment of chosen areas of each color file, will produce graphic results similar to intensity emission bands graphics, which will facilitate to make a ratio between data of the channel red against green and obtain similar results to experimental ones.

The application presented here is based on computer software, to apply the mentioned program in the smartphone to directly obtain visual data and at the same time treat it for thermal sensing purposes, MATLAB offers a visual treatment data called Simulink [24], which connects the camera of an electronic device to the MATLAB software and extract the visual data as input for the mentioned program.

Due to external events and time limitation, the application approach is presented in the supporting information and not as a confirmed result in the following segment.

3. RESULTS AND DISCUSSION

3.1 Selection of a biological specimen

The chosen plants for these experiments are common lentils, *lentis culinaris*. The reasoning behind this selection is that lentils are part of a big family of plants called *Fabaceae*, which are part of the angiosperms in the *Plantae* kingdom [19]. The *Fabecae* or *Leguminosae* family are commonly known as legumes, one of the biggest dietary source for humans [20] and one of the three largest land plant families, together with *Orchidaceae* and *Asteraceae* [19]. The large number of specimens in this family of plants makes it suitable to choose one of them and study their behavior under manipulated temperature environmental conditions. The obtained results can be used as a model for the rest of the land plant families.

3.2 Lentil plant specimen growth

The growth of the lentil seedling specimens, and watering them with particles colloidal dispersions with different concentrations ranging from 0.01 to 0.05 $\frac{\mu g}{mL}$ can be seen in the Figure 1.

For control purposes, a control plant sample (on the left in each row), in which no NPs were used when watering them, was used. The results showed no effect on the development of the seedlings when watered with the NPs dispersion. The sample watered with a $NaYF_4$ NPs concentration of 0.05$\frac{\mu g}{mL}$, the maximum used in this study, showed a similar growth rate than the control plant sample, in agreement with previous results in bibliography, in which was stated that this particles concentration should not affect the biological specimen [2].

The only adverse effect due to the UCNPs watering to the lentil plants samples is shown in the left of figure 3. It can be observed that the tap root for that specimen showed evidences of drying, when watered with the colloidal dispersion. Reasoning behind this statement is that the particles are commercially produced for

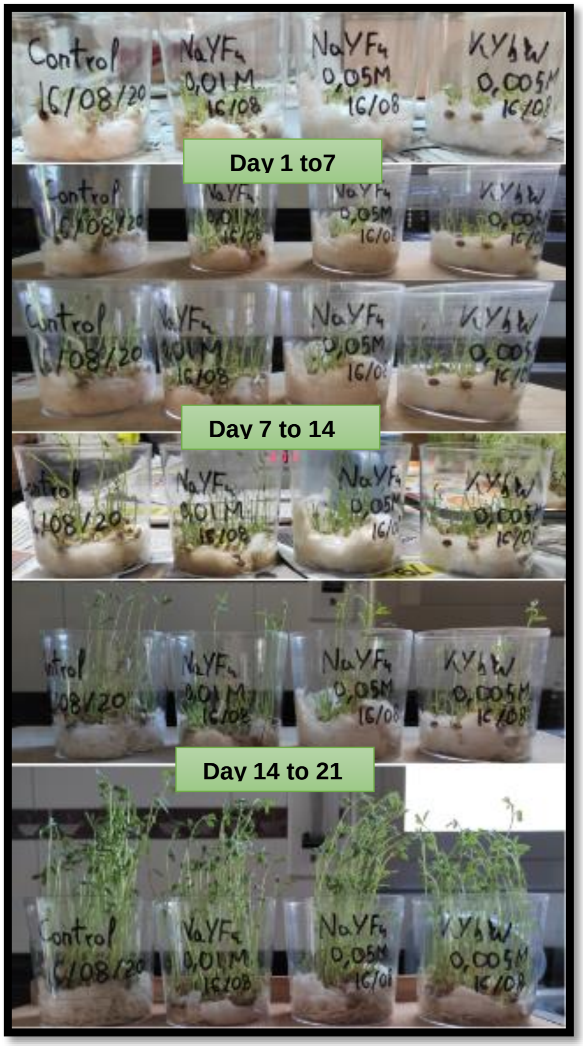

Figure 1 Time-lapse of plant samples growth following the method of watering with Er,Yb:$NaYF_4$ colloidal dispersion

luminescence purposes, and their size dispersion covers the range from nanometers to micrometers, and even forms conglomerates. The sizes in the micrometers range are too large for the present application [2], and only the smaller nanoparticles can be absorbed by the plants. The rest of the particles cover the surfaces of the roots, drying them after 15 days, because the roots could not absorb water and nutrients anymore due to the particles blocking their hair roots.
As previously stated lentils are part of the *Leguminoseae* family, and one the main features of them is the primary root system, which is based on a first grown or principal root that connects with the radicle, and from that a root system that grows from it, constituting a tap root system, as shown in the figure 2.

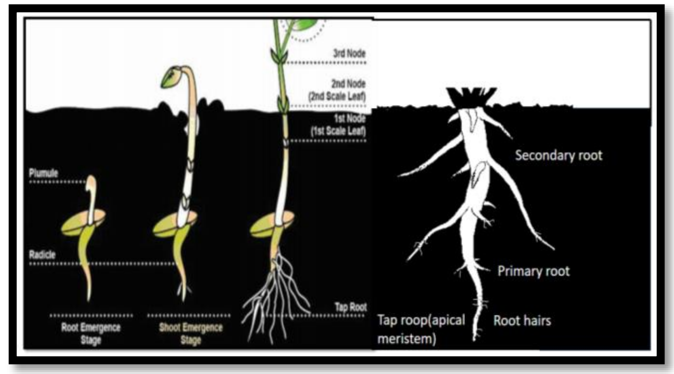

Figure 2 Development stages of a lentil root system in soil. Adapted from Parts of the root system. https://www.legumematrix.com/images/563/Lentil_Manual_Saskatchewan.pdf>

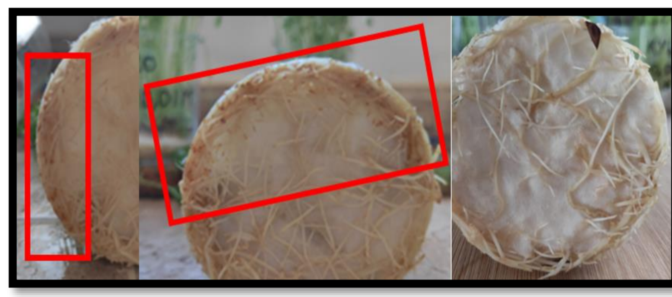

Figure 3 Left; Effects of root drying due to $NaYF_4$ watering. Middle; similar drying effect with KYbW watering, with similar particle size. Left: Control Sample unaffected

The growth of a secondary root produces the appearance of root hairs. Those will be the agents in charge to absorb any external agent that can be considered as nutrient. As a result, the absorption capacity of a plant is equal to the surface area of the secondary root system. So that, a bigger number of secondary roots, will increase the absorption rates of water and nutrients, including the UCNPs with which we watered them, and making easier their internalization in the internal system of the plant, known as xylem [21]. For this reason, lentil plants, which produce a tap root (apical meristem) composed by a large number of secondary roots.

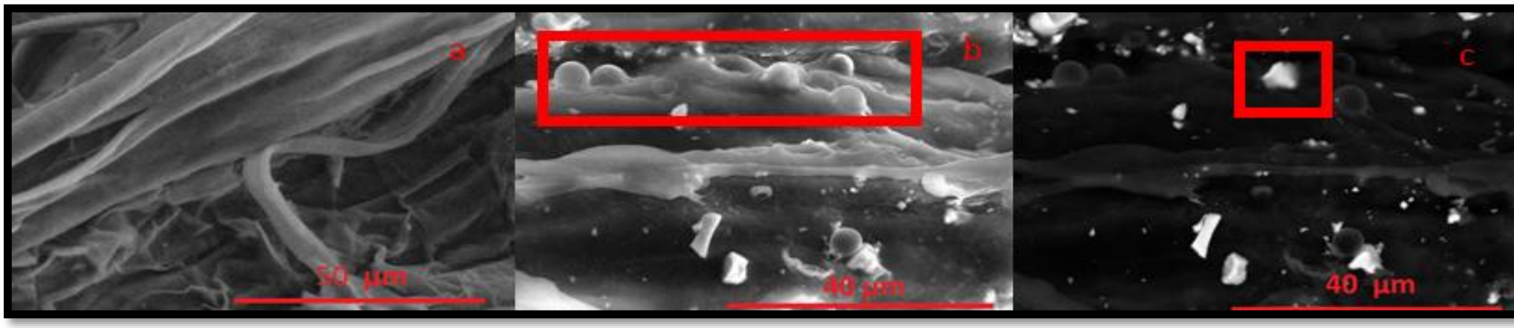

Figure 4. a) ESEM image of a common secondary lentil root b) ESEM image of a lentil root watered with $NaYF_4$ c) backscattering image of figure 4.b

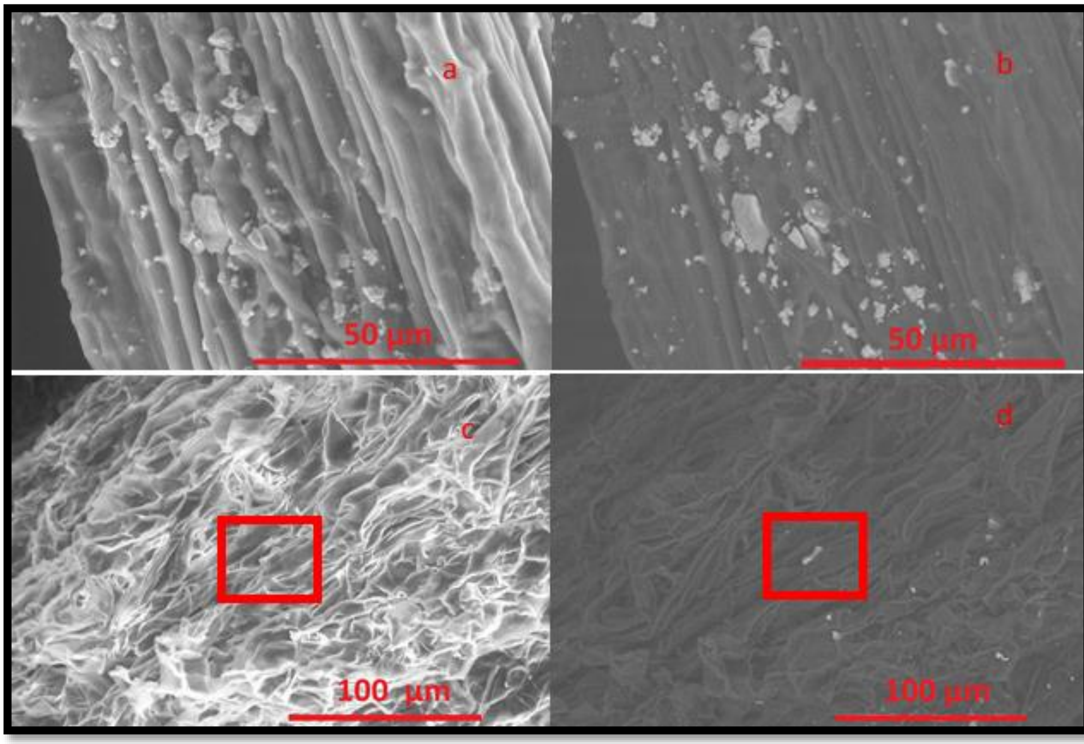

Figure 5 a) ESEM picture for an ultra-sounded root b) backscattering image for Er,Yb:$NaYF_4$ presence confirmation c) ESEM image for a lentil plant root opened in half d) backscattering image of picture c that shows presence of Er,Yb:$NaYF_4$ material.

3.3 Surface analysis of the lentil roots

Figure 4 shows SEM images of the roots of a control lentil seedling (Figure 4 (a)), and the roots of a lentil seedling after being watered with Er,Yb:$NaYF_4$ particle water dispersion (Figure 4 (b).

The appearance of protuberances (indicated by the red mark) in the roots watered with NPs suggests that they are internalized by the roots into their xylem system, as has been observed in previous works [22]. Figure 5(c) corresponds to the same root area than Figure 5 (b), but recorded with backscattered electrons. It shows that there is a difference in the contrast of the image between the root surface and the protuberances, indicating that they are composed by different elements. An Energy dispersive Spectroscopy (EDS) analysis indicated that these protuberances contain Yttrium and Ytterbium in their elemental composition, indicating that Er,Yb:$NaYF_4$ nanoparticles are internalized in the xylem of the lentil seedlings (Figure 2 in the supporting information)

Up to this point, the ESEM images have only shown the external part of the root.

To confirm the internalization of the Er,Yb:$NaYF_4$ in the xylem system, mainly found in the primary root , the lentil root was cut-open with an scalp, and observed under the ESEM microscope. Figure 5, shows the result of this characterization. Here the presence of NPs is more evident. The backscattered electrons image, and the EDS analysis confirm these results. The results leads to visible possibility that Er,Yb:$NaYF_4$ NPs can be internalized as was stated in the bibliography [2], but in this case for lentil seedlings. Being also the case that the amount of NPs in the outer parts of the tap root (figure 4 (b)) is far less that what can be in the interior parts of the root system (figure 4 (c) And (d)) also provides an idea that the luminescence spectrum that will be obtained, is going to be mainly related to internal temperature.

3.3.1 Particle localization under luminescence observation

By observing the visible luminescence of the Er,Yb:$NaYF_4$ particles excited with the NIR, we visualized the distribution of UCNPs alongside a root specimen. Figure 6 (a) shows a photographic composition of the tap root system, constructed from different images recorded with the excitation laser focused on specific points. Figure 6 (b) shows a composition of images depicting a secondary root including the tap root. In both pictures it can be observed the presence of the UCNPs in two main zones: (i) near the root tap, given that the main development of the root happens here seems to be the zone which will absorb more concentration of nutrients (or NPs in this case); (ii) near root hairs due to being the main absorption tool of the root. Most of the NPs are found near the tap root, even the plants were watered with the Er,Yb:$NaYF_4$ particle dispersion 10 hours before the experiment.
In a previous work it was stated that the NPs could be absorbed and transported to the radicle and even further in only 3 hours [22].
The slow transport of the Er,Yb:$NaYF_4$ nanoparticles alongside the xylem system in our lentils seedlings could be related to the large size of the particles. So, it can be concluded from this section, that the presence of the Er,Yb:$NaYF_4$ particles in the root system is enough for thermometric measurements.

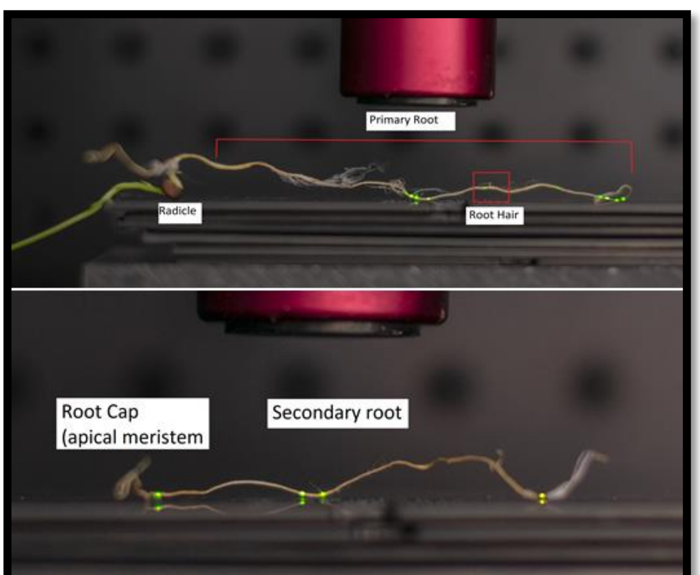

Figure 6. Photographic composition of a) a tap root system, and b) a secondary root under the excitation with a 980nm laser source

3.4 Luminescent thermometry

Being the temperature sensing the objective of this work, the knowledge of the evolution of the luminescent bands intensity, produced by the UCNPs that are internalized by the biological system, in relation with temperature is due.We have chosen an RGB (red-green-blue) approach for this analytical treatment due to the limited spectral resolution of a smartphone camera, which makes it unable to differ values between close emissions, in terms of wavelength values, in the visible spectrum. So, they have been selected the green range (525-550 nm) and the red range (620-700 nm) to integrate the photoluminescence band intensity from the Er,Yb:$NaYF_4$ particles [5].
The thermal sensing using a smartphone camera has been explored with an RGB approach in previous bibliography [26]. A thermal calibration of the intensity of the emissions of the UCNPs will be done with the purpose of collecting proper analytical data to produce an optimal model for thermal sensing.
The emission bans of this work are 520 nm ($^2H_{11/2} \rightarrow {}^4I_{15/2}$), 545 nm ($^4S_{3/2} \rightarrow {}^4I_{15/2}$) and 650 nm ($^4F_{9/2} \rightarrow {}^4I_{15/2}$). The mechanism by what those bands appear is due to Erbium and Ytterbium elements present in the crystalline structure of the $NaYF_4$, when the material is excited with a 980 nm laser source the up-conversion mechanism will produce luminescence, the stated emission bands, due to a radiative decay. The mechanism is shown in the figure 7 and figure 8 shows the room temperature up-conversion spectra for the Er,Yb:$NaYF_4$ particles under 980 nm excitation.

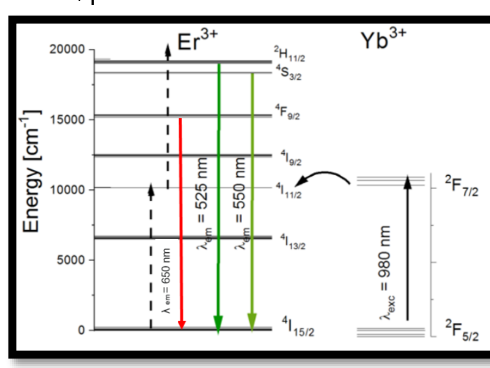

Figure 7. Scheme for up-conversion process

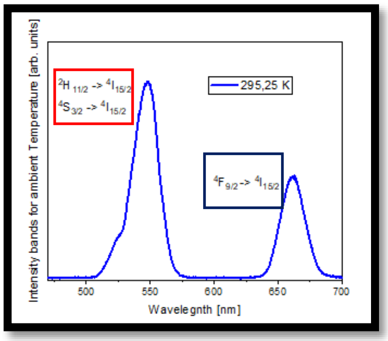

Figure 8 Ambient temperature emission bands for Er,Yb:$NaYF_4$.

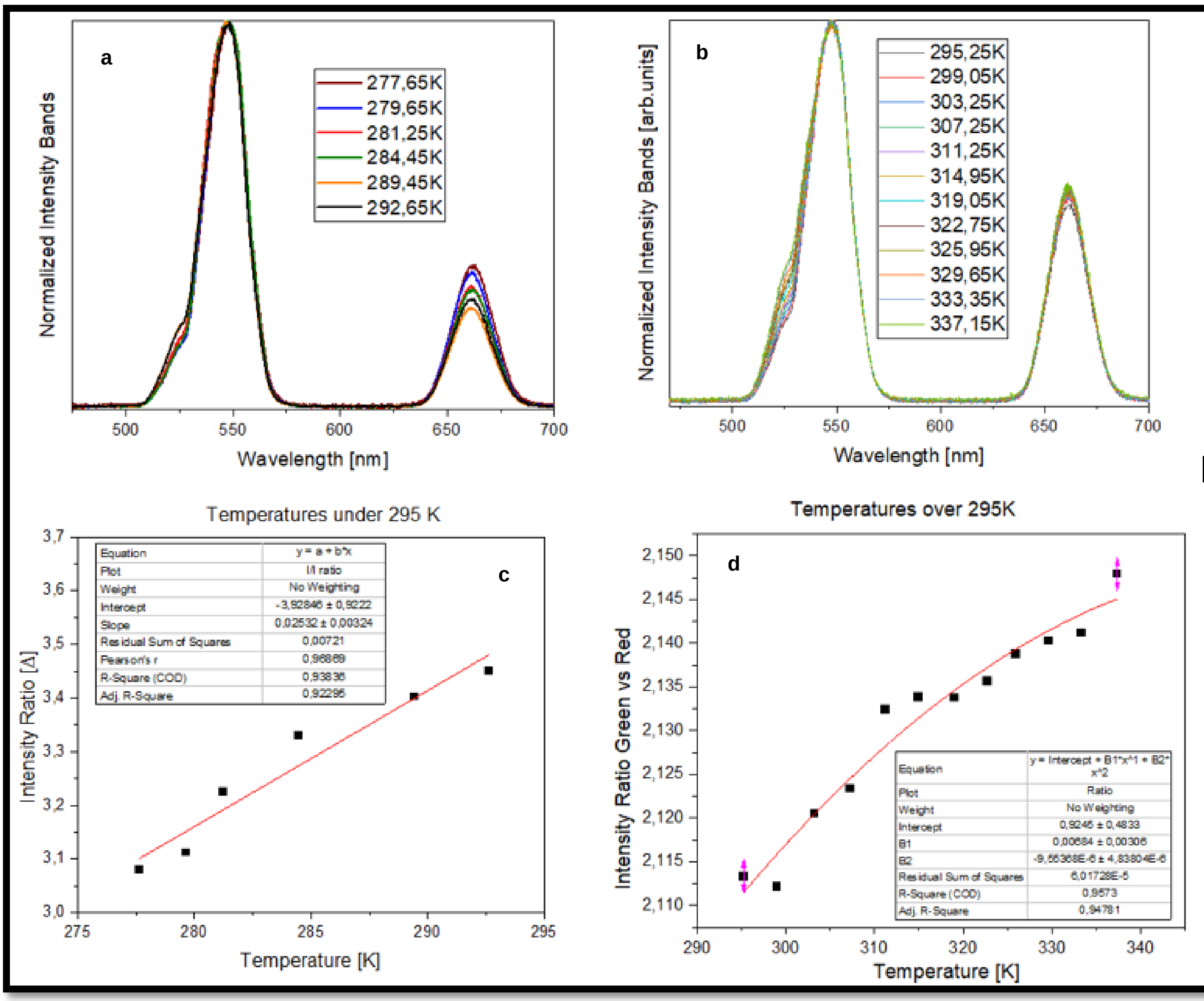

Figure 9 (a) Photoluminescence emissions of Er,Yb:$NaYF_4$ UCNPs, recorded in microlum set-up excited by a 980 nm laser source from 279 to 295K and normalized by the maximum green intensity at 548 nm(b) Photoluminescence emission of Er,Yb:$NaYF_4$ UCNPs, recorded in microlum set-up excited by a 980 nm laser source from 295 to 335 K and normalized by the maximum green intensity at 548 nm (c) Thermometric calibration of the selected intensities for the temperature range of 279 to 295 K (d) Thermometric calibration of the selected intensities for the temperature range of 295 to 335 K.

Figure 9 shows the variation of intensity of the luminescence bands of the UCNPs with temperature, after being excited at 980 nm, in relation to the increase of temperature from 273to 335 K. The spectra obtained from figure 9 will be used to extract the calibration curves, have been divided in two different graphs since two different set-ups were used to collect them, depending if the calibration was performed for temperatures below room temperature, or above this temperature, as explained in the Experimental Section. Figure 9 (a) and (b) show the normalization of the emission bands in respect to the maximum intensity value of the green emission, relates to the ${}^4S_{3/2} \rightarrow {}^4I_{15/2}$ transition, the objective is to show the variation of the ${}^4F_{9/2} \rightarrow {}^4I_{15/2}$ transition

We calculated the intensity ratio as $I_{550}/_{660}$. Figure 9 c and d show the evolution of the calculated intensity ratios with temperature again in two different graphs according to the set-ups with which the spectra were recorded. The evolution of these intensity ratios were fitted phenomenologically to an equation following a linear tendency (R= 0.9230) for temperatures below room temperature (see Figure 9(a)) and a second order polynomial equation (R= 0.9478) for temperatures above room temperature (see Figure 9(b). The obtained equations are listed in Table 1. These expressions will be used to determine the internal temperatures of the lentils seedlings roots.

Table 1 Equations that result from the thermometric calibration of the green/red emission integrated intensity ratios and their variation with the temperature using Er,Yb:$NaYF_4$r particles excited at 980 nm.

Thermometric calibration of Er,Yb:$NaYF_4$	
Temperatures under 295 K	Δ= 0.02532 – 3.92846T
Temperatures over 295 K	Δ = 0.9245+0.0684T - 9.55368T^2

The ability of the Er,Yb:$NaYF_4$ NPs to be used as luminescent thermometers is obtained from the knowledge of the variation of the intensity ratio obtained for a small change of temperature. This is commonly done by calculating the absolute thermal sensitivity (S_{abs}) from the first derivative of the intensity ratio to respect the temperature:

$$S_{abs} = \frac{\delta\Delta}{\delta T} \qquad (1)$$

Nevertheless, S_{abs} depends on the set-up used to collect the spectra, and hampers the comparison with other thermometers based on spectra collected with a different set-up, or even with other thermometers based on different techniques. A normal quantification of the thermal behavior for this type of thermometers is done with the relative thermal sensitivity (S_r) which is obtained from the function:

$$S_r = \frac{1}{\Delta}\frac{\delta\Delta}{\delta T} \qquad (2)$$

In the case for the UCNPs in the project this relative sensitivity was calculated for the range of 275-295K giving a result of 0.35%/K and for the range of 295-335K giving a result of 0.32%/K. Analytical graphics for all the data in the temperatures ranges can be found in the supporting information. The following table shows comparative from the nanoparticles in the project against others nanothermometers that work in that range of temperatures. The results are showed as a graphic in figure 3 of supporting information.

The resolution of temperature (δT) is calculated as:

$$\delta T = \frac{1}{S_r}\frac{\delta\Delta}{\Delta} \qquad (3)$$

The results are showed as a graphic in figure 5 of supporting information.

Table 2 Information for the S_{rel} of the particles in the ranges of this study and of the bibliography

Nanoparticles	S_{rel} [%*K^{-1}]	Temperature [K]
Er,Yb:$NaYF_4$: *	0.35	275-295
Er,Yb:NdF_3: [27]	0.26	285-335K
$NaYF_4$:Yb, Er*	0.32	295-335
Er,Yb: $KMnF_3$ [26]	5.7	303-343K
Tm/Yb:CaF_2 [27]	0.25	298-333K

3.4.1 Temperature sensing inside the lentil seedlings roots

Temperature measurements inside the lentil seedlings roots were done over a 3-day span. The environmental temperature at which the plants were exposed can be found in Table 2 in the supporting information. Those will be the reference values regarding the plants ability to thermally regulate themselves [14] or on the contrary that they have the same temperature as the environment.
Figure 10 shows the spectra recorded out of the luminescent particles internalized in the lentil seedlings roots under excitation at 980 nm. The RGB approach was used to obtain the thermometric values (shown in the column as I_{550}/I_{660}), given that the data used is separated into two color emissions (green and red) it can after be used for the calibration using as a detector and smartphone. The values obtained from it are shown in the following table 3.

The results that can be seen from Table 3 is that thermal values obtained from the internal structure of the lentil seedlings roots differs from the thermal values obtained from their environment. The degree of variance is not that extreme, in colder temperatures the thermal values of the roots show to be 1.3±0.6ºC different from the external, this could be associated to grade of difficulty in terms of thermoregulation [14]. Meanwhile at ambient temperature or higher temperatures the plant is able to reduce its temperature more efficiently.

Day of data collection	Environment (ºC)	I_{550}/I_{660}	Calculated temperature Values (ºC)
Day 1	Ambient (19.5)	2.099	20.1
	Cold (16.5)	3.388	15.8
	Hot (32.5)	2.116	28.1
Day 2	Ambient (20.8)	2.102	21.6
	Cold (13.6)	3.361	14.9
	Hot (29)	2.114	26.7
Day 3	Ambient (22.3)	2.110	22.1
	Cold (12.7)	3.358	14.6
	Hot (29.6)	3.894	-

Table 3 Experimental Data for temperature correspondence between outer environmental values and internal temperatures measured in the lentil seedlings roots

The experimental results from this section show physical evidences that Er,Yb:$NaYF_4$ particles can be used as an internal nanothermometer for seedlings of the *Leguminosae* family, given that the photoluminescence measured comes from the particles absorbed by the biological system. From the experimental measurements shown here, only the higher temperature in the third day showed an outlier behavior.

4. Conclusions

In summary this work performed an study of the internalization of the Er,Yb:$NaYF_4$ particles in lentil seedlings, based on watering by a colloidal dispersion. The study supported by ESEM analysis showed visual example of the particle internalization in the roots of the plants. The thermal characterization of the particles by an RBG approach showed promising results, being their sensitivity 0.35%/K in the 275-295 K range and 0.32%/K in the 295-333K range.

From an application stand-point the ability to produce an internal thermometer shows promise in the crop growth and development field of study, because as it was stated little temperature changes can drastically effect the crop results. For that reason the ability of this UCNPs to be a thermal sensor for the internal temperature of the plants, could facilitate field work due to knowing the if the specimen is in the desired range of temperature for their development or not.

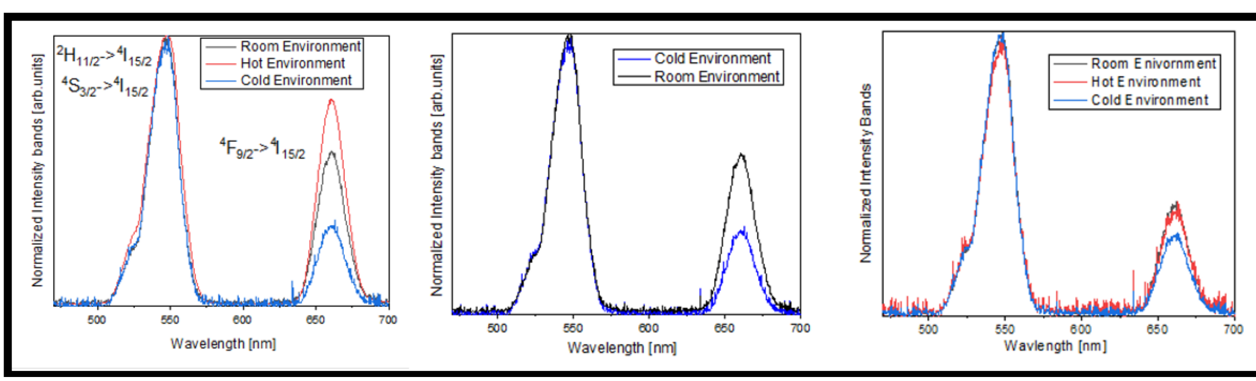

Figure 10. Photoluminescence band emissions from the UCNPs internalized by the root system

5. Acknowledgments

Thanks to FiCMA-FiCNA research group for support and assistance. To Dra. Cinta Pujol and Dr. Joan Carvajal for their help in these strange times and especially to Nicolette Bakker for their support as technician during the project. Finally thanks to Alex Moya, for their help in the photographic segment of the lentil roots.

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Supplementary Information

Table 1. Resume table of the growing method for lentil plants watered with Er,Yb:$NaYF_4$ colloidal dispersion

	Day 1 to 7/8	Day 7/8 to 10/14	Day 10/14 to 21	Day 21 to plant decease
Watering solution	Downlayer:5 mL Water Top layer: 5 mL Water	Top Layer: 15 mL Water	Top Layers: 10 mL Bottom with syringe: 10 mL of Water + 0.5 $\frac{\mu g}{mL}$ $NaYF_4$	Bottom with syringe: 30 mL of Water + 0.5 $\frac{\mu g}{mL}$ $NaYF_4$ or only 30 mL of water
Considerations	Only watering, to ensure that the lentil seed is humidified with purpose of development start	Similar purpose with the first week.	Here the intention is to ensure the humidified atmosphere and directly make the NPs contact with roots.	Here the choosing can differ due to the state of the roots, and analysis needs to be made.

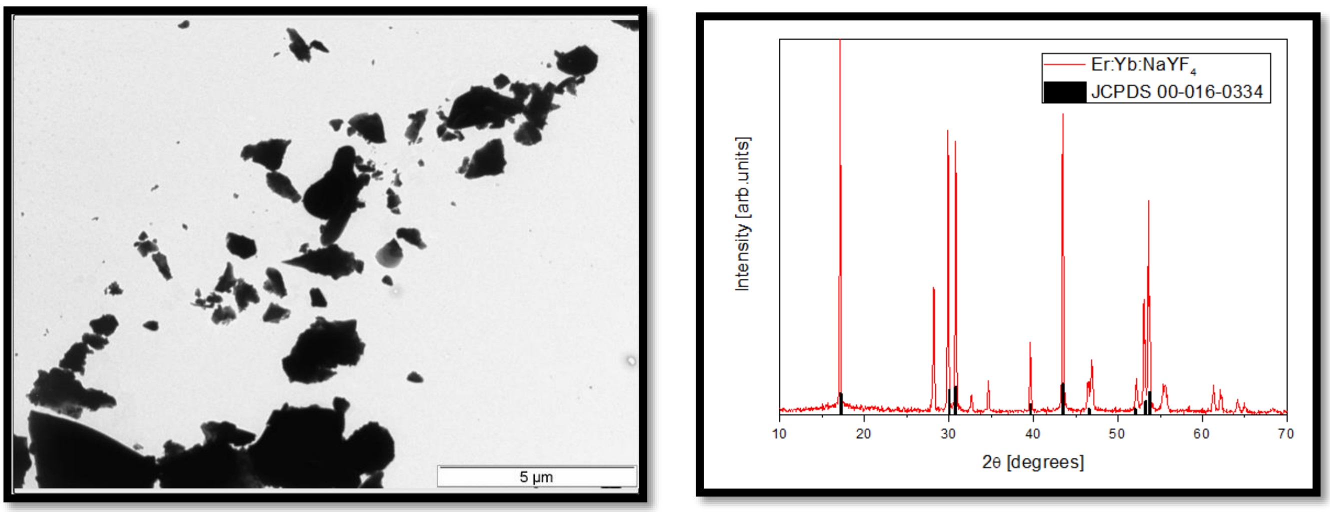

Figure 1 TEM images of the commercial Er, Yb:$NaYF_4$:particles and their XRD analysis

Element	Weight%	Atomic%
C K	30.36	51.33
O K	8.50	10.79
F K	27.04	28.90
Na K	3.10	2.74
Y L	23.46	5.36
Yb L	7.53	0.88
Totals	100.00	

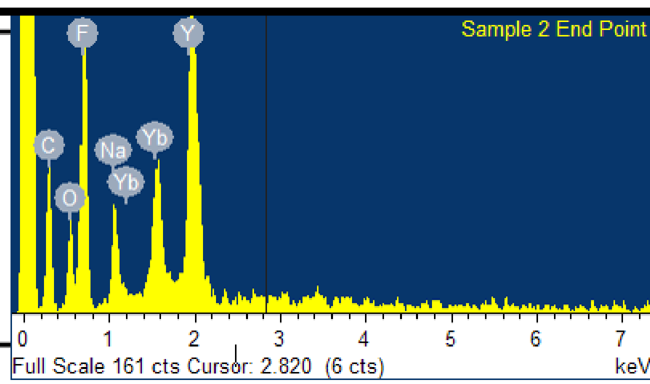

Figure 2.EDS analysis of the ESEM image of the protuberances in the lentil plants roots

The following tables show values of the thermal measurements held in the resting place where the plants were conserved, and the moment before doing the luminescence detection in the experimental room.

Table 2 Experimental measurement of external lentil root temperature

06/08/20		Resting placement		Set-up Microlum	
		Infrared	Thermocouple	Infrared	Thermocouple
Ambient		21 ºC	23.3 ºC	18.8 ºC	19.5 ºC
Cold	Root sample 1	3ºC	8.5 ºC	15.5 ºC	16.5 ºC
	Root sample 2	4.7 ºC	8.7 ºC	16.1 ºC	17.3 ºC
Hot	Sample 1	36.7 ºC	34.3 ºC	31.3 ºC	32.5 ºC
	Sample 2	36.7 ºC	33.7 ºC	30.8 ºC	31.7 ºC

* Sample 2 was not a watered plant, it was a common lentil plant just for confirmation purposes, and to confirm that there were not extraordinary thermal values.

Table 3 Experimental measurement of external lentil root temperature

07/08/20		Resting placement		Set-up Microlum	
		Infrared	Thermocouple	Infrared	Thermocouple
Ambient	Sample 1	20.8 ºC	23.4 ºC	20.6 ºC	20.8 ºC
	Sample 2			21.9 ªC	22.3 ªC
Cold	Sample 1	3.8ºC	8.9 ºC	13.2 ºC	13.6 ºC
	Sample 2	8.7 ºC	9.1 ºC	12.7 ºC	13.6 ºC
Hot	Sample 1	43.2 ºC	38.7 ºC	30.6ºC	29 ºC
	Sample 2	42.7 ºC	37.9 ºC	30.8 ºC	30.1 ºC

Table 4 Experimental measurement of external lentil root temperature

07/08/20		Resting placement		Set-up Microlum	
		Infrared	Thermocouple	Infrared	Thermocouple
Ambient	Sample 1	20.7 ºC	24.1ºC	21.8 ºC	22.3 ºC
	Sample 2			22.2ªC	22.9 ªC
Cold	Sample 1	7.4ºC	11.9 ºC	11.9 ºC	12.7ºC
	Sample 2	9.6 ºC	9.7 ºC	13.3ºC	13.9 ºC
Hot	Sample 1	33.9 ºC	30.9ºC	30.8ºC	29.6 ºC
	Sample 2	33.9 ºC	32.3 ºC	31.2ºC	30.7 ºC

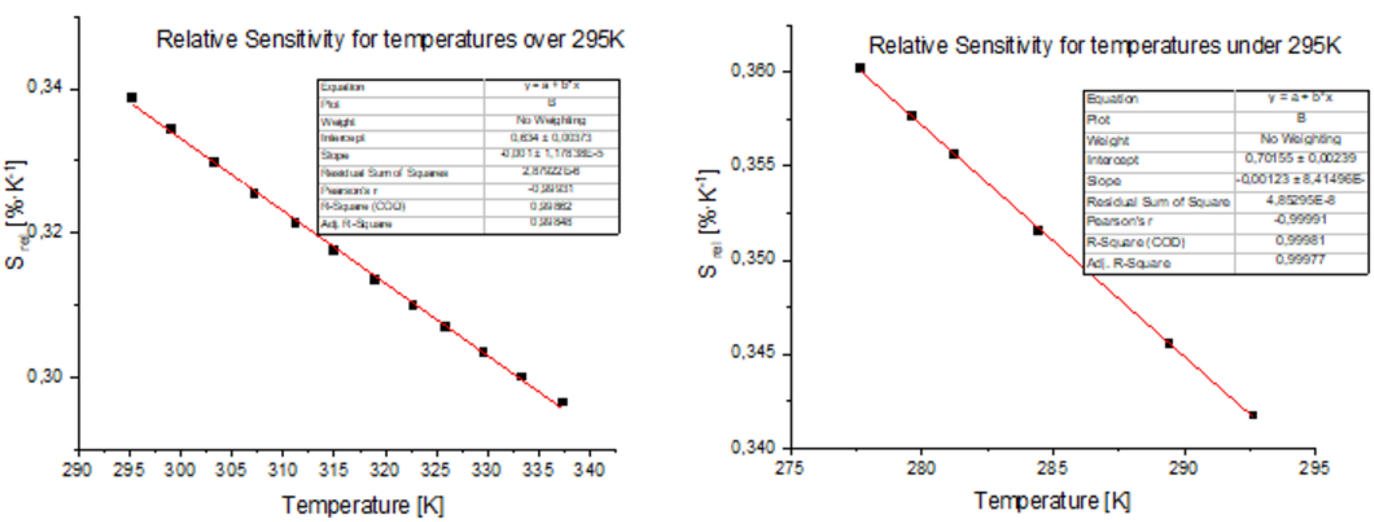

Figure 3 Relative Sensitivity for both experimental temperature ranges

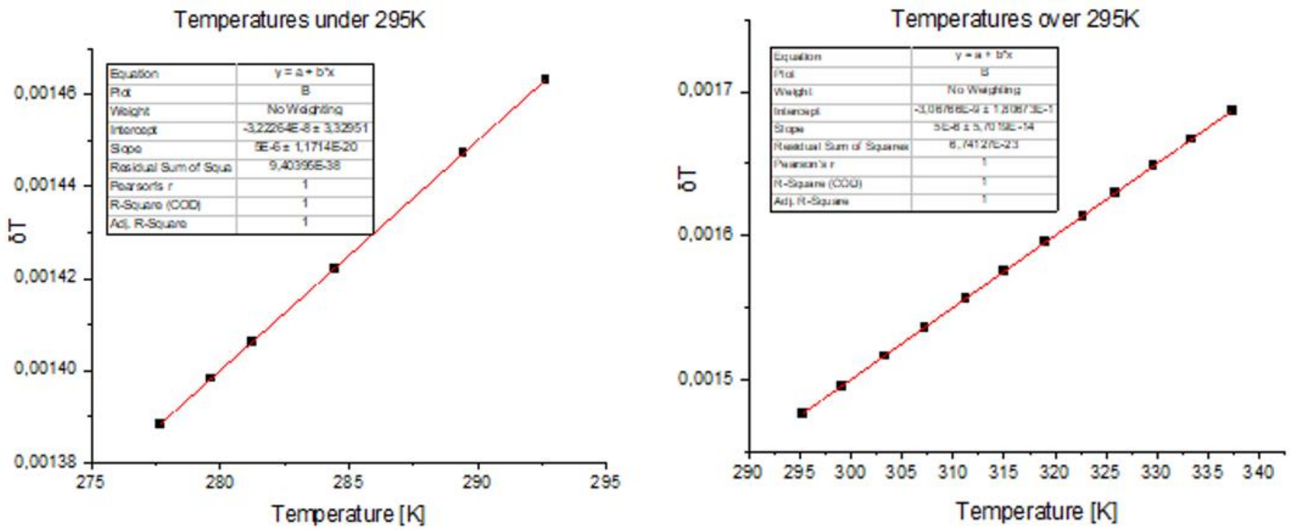

Figure 4 Temperature resolution for both experimental temperature ranges

Development of a thermometric sensing application for smartphones based on Er,Yb:NaYF$_4$ emission

The luminescence data obtained in the previous section using the Ocean Flame spectrophotometer was here recorded with a camera from a smartphone. Figure X and Figure X, show the visible emission generated by the NaYF4 particles recorded with the Xiaomi Mi Lite 10 and the Xiaomi Redmi 4 smartphones, being quite similar. This similarity is due to the fact that the maximum spectral resolution comes from the camera detector, and in the case of both smartphones (and almost all currently used smartphones) those are RGB detectors [17]. The ability of color filtering in a smartphone software is related to the ability of their RGB detectors to separate the colors as much as possible, while the clarity of a picture in their raw data version is related to the pixel/nm ratio (ref). In conclusion even though a smartphone with more pixel/nm ratio will be more precise in principle, the temperature sensing ability will not be influenced by this parameter.

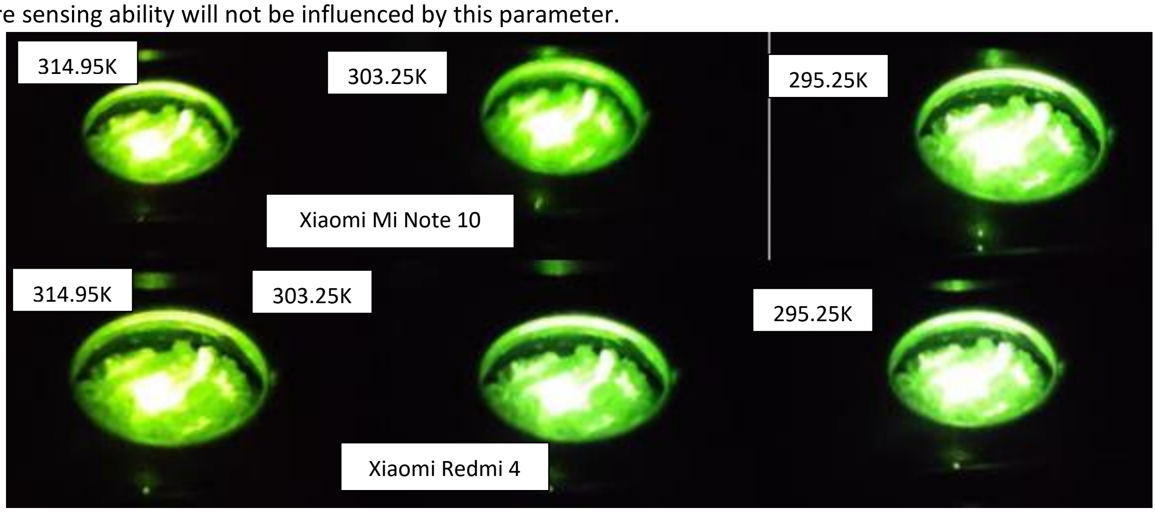

Figure 1 Image data obtained by a Xiaomi Mi Lite 10 (upper row) and Xiaomi Redmi 4; from left to right an increase of pure green emission can be observed in relation to temperature decrease. The Er,Yb:NaYF$_4$ commercial powder particles were excited with a 980 nm laser source.

With the aim to obtain a thermometric calibration through a RGB approach using a smartphone as detector, the variation of the intensity of the whole luminescence emission was photographed at different temperatures. The pictures were recorded at the same temperatures at which the spectra of the nanoparticles were collected with the Ocean Flame spectrophotometer for the calibration of the luminescent thermometers, as explained in the previous section. The visual photoluminescence seen here is basically green. This is due to the brighter emission in the green part of the visible spectra generated by the nanoparticles, but also because human eyes are more sensible to green light, which overcame other visible emissions produced [4]. This is not a limiting factor, because even though visually the photograph is not comparable with the spectra obtained by a spectrometer, the raw data that can be obtained from the image data (photography) it has also the data from the emission of the red part of the visible spectrum

MATLAB software has been used for this purpose. The procedure is the following: every visual input can be read with the function "rgbImage" ; which is a function able to read images an convert it into raw data for the software, so then can be treated. Then this software is able to divide the data into the 3 color channels that the smartphone uses to produce the photographic result. This can be done using the function "redChannel=rgbImage(:,:,1)", and doing the same for Blue and Green channels. By this, three archives are created, all of them being the original photo in a grey scale (using function rgb2gray) but separated based on the color participation. Figure X shows the result of this separation for one of the images collected with the smartphone, where it can be seen clearly that the intensity in the red channel is clearly lower than that of the green channel, in agreement with the intensity of the green and red bands collected with the Ocean Flame spectrophotometer. The change of intensities and color observed in these pictures is enough to confirm that thermometric measurements can be done by smartphone as it was previously done by change in QR color in a previous study [25]. At this point, the intensity can be extracted from a specific area of the image, knowing the row and columns limiting the area by using the formula "meanGrayLevel", which gives the mean value of a rectangular chunk of the grey scale image. The resultant mean values are between 0 and 255, which is obtained of an area could produce an "Intensity pattern", or in other words a emission graphic, given the desired values. This could be translated to what the project has been using as input, the emission bands graphics, and be able to apply the RGB approach to obtain thermal values.

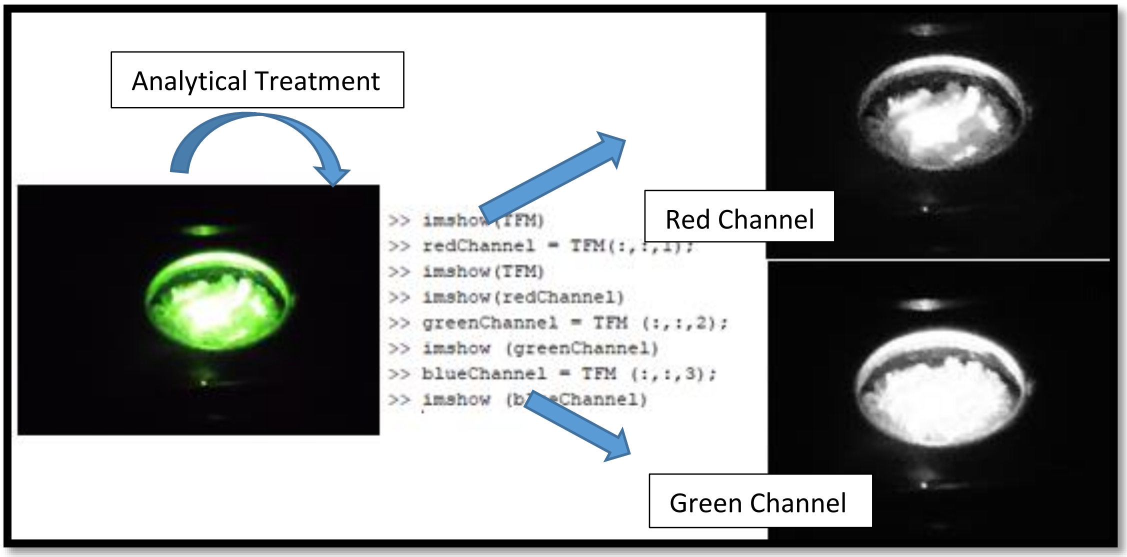

Figure 2 Brief introduction into grey scale results from a Raw Data. Observe that the grey scale is darker in the Red Channel due to low presence of the color in the initial picture, while in the Green Channel almost all of the Luminescent zone is white

The limiting factor of this method, is related to the detector data, when done experimentally the graphics are obtained by an average time, meaning the time that the detector will be recording data from the observed source. While data obtained from a smartphone, is an instant picture, not data obtained over time as the detectors. This as previous bibliography states [25], makes the treatment of photographic data not comparable with data obtained with a detector. The approach proposed here to overcome this problem, is to use the MATLAB tool, Simulink Support Package [24], this tool makes possible to use the smartphone camera as the original source of data, instead of a picture taken. This will provide the needed over-time data, which now will be continuous frames, for analytical treatment comparable to what was done in the previous section to measure the thermal values in the internal structure of lentil plant roots. Finally if this could be done, a thermal calibration of the particles as has been done in this project, will be needed in order to confirm comparable results, meaning that thermal values obtained from this approach are equal to values obtained from the laboratory calibration.

The final version of an smartphone only dependent application, will be done by using MATLAB function block [24], where the code could be embed and transported to and Android System Library where and application can be created. Another barrier for the application is that by the moment this is explained, the function to use an application based on MATLAB Simulink is not viable, but is stated by the company that should be able in the following fiscal year.