A framework for far-field infrared absorption microscopy beyond the diffraction limit

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Abstract: A framework is proposed for infrared (IR) absorption microscopy in the far-field with a spatial resolution below the diffraction limit. The sub-diffraction resolution is achieved by pumping a transient contrast in the population of a selected vibrational mode with IR pulses that exhibit alternating central minima and maxima, and by probing the corresponding absorbance at the same wavelength with adequately delayed Gaussian pulses. Simulations have been carried out on the basis of empirical parameters emulating patterned thin films of octadecyltrichlorosilane and a resolution of 250 nm was found when probing the CH₂ stretches at 3.5 μm with pump energies less than ten times the vibrational saturation threshold.

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OCIS codes: (170.0180) Microscopy; (300.1030) Absorption; (300.6340) Spectroscopy, infrared; (300.6500) Spectroscopy, time-resolved; (300.6360) Spectroscopy, laser; (300.6390) Spectroscopy, molecular.

References and links


1. Introduction

Developments in biomedical and nano-materials’ research rely increasingly on state-of-the-art instruments capable of structural imaging and spectral analysis at sub-cellarular and nanometer resolution. Because vibrational modes of molecules are dependent on chemical bonds and local molecular arrangements, vibrational spectroscopies reveal molecular fingerprints, the mapping of which produces label-free imaging modalities, such as coherent anti-Stokes Raman spectroscopy (CARS) [1–6], stimulated Raman scattering [7], spontaneous Raman [8,9], and Fourier-transform infrared absorption spectroscopy (IRAS) [10–19]. However, the spatial resolution of these methods is restricted by diffraction to values between λ and λ/2. This imposes a severe limitation especially for IRAS given that in the mid-infrared region the wavelength λ spans from 3 to 20 µm. The resolution for IRAS imaging in the far-field thus remains poor when compared to the resolution that can be achieved using near-field scanning.
probe approaches (50-100 nm) [20–22]. Conversely, the latter techniques suffer from practical limitations arising from the scanning of a material probe a few nanometers above the surface of the sample. Achieving high resolution in far-field imaging thus remains of prime interest.

Nanoscale and sub-diffraction resolution have been extensively demonstrated in far-field fluorescence microscopy [23], with reversible optical fluorescence transition (RESOLFT) [24–31], non-linear structured illumination [32,33], and single molecule localization microscopies [34,35]. However, these methods generally require the introduction of fluorescent markers in the samples and do not provide label-free local chemical information. Novel chemical imaging tools with high resolution are thus sought for and, in view of that, schemes for sub-diffraction vibrational microscopies based on far-field CARS have been proposed [36,37]. For these schemes, it is envisaged to control the spatial extension of the measured sample volume by suppressing the vibrational Raman coherence through an incoherent process involving the excitation of a neighboring vibration [36] or by periodically depleting the ground-state through Rabi oscillations [37]. These concepts are expected to improve the spatial resolution down to ca. 65 nm [37], which would represent a breakthrough in CARS microscopy.

In this paper, we discuss and model a framework for IRAS imaging with spatial resolution beyond the diffraction limit. In the proposed methodology, the effective point-spread function (PSF) is confined by generating a spatial contrast in the depletion of the ground-state of a vibrational mode of interest using a scheme of IR pumping and IR probing, which results in a PSF proportional to the local IR absorbance. Here below, the method is termed as vibrational-depletion IR (VD-IR) microscopy, and it is illustrated by simulating PSF and images of patterned thin films of octadecyltrichlorosilane adsorbed on an IR transparent dielectric substrate.

2. Vibrational depletion IR microscopy

Figure 1(a) illustrates the concepts of IR absorption and pump-probe spectroscopy [38–40], where both pump and probe pulses are tuned to the wavelength of a vibrational transition. A pulse of low intensity undergoes partial absorption through a sample of two level oscillators and the absorption of each IR photon leads to the excitation of one oscillator from the ground state $|0\rangle$ to the excited state $|1\rangle$ (left). The competition between stimulated absorption and emission processes prevents the population of $|1\rangle$ to exceed that of $|0\rangle$, so that a pulse of very high intensity excites at most half the oscillators, leading to the vibrational saturation of the sample (middle). In the pump-probe experiment, the same pulse of very high intensity is immediately followed by a delayed probe pulse whose absorption is reduced, for delays shorter than the lifetime of $|1\rangle$ (right). In other words, this second pulse probes the ground-state population in the sample and regions of the sample that have been irradiated by the pump are virtually transparent to the probe for short delays, where the ground-state remains depleted.

A suitable PSF for VD-IR imaging is realized by alternating the spatial definition of the pump, by using a first one characterized by a central anti-node and a second by a central node, as shown in Fig. 1(b). The absorption of the probe is minimized or nullified over its entire spatial extension in the first case, thus creating a reference signal, and absorption of the probe is confined to the nodal region of the pump in the second case. The difference in integrated pulse intensity gives the probe absorption confined to the center of the nodal pump, where the sample population $|1\rangle$ remains below saturation, and is defined here as the VD-IR signal. Increase in pump intensities reduces the spatial extension of the unsaturated center and thus increases further the localization of the VD-IR PSF, since sample regions that are saturated for both pump patterns do not contribute to the differential signal.

Although various geometry are possible, the spatial distribution of the nodal pump intensity is set to that of a first-order Gaussian which can be readily generated passing a zeroth-order Gaussian beam through a vortex phase plate [41–44]. The latter modify the phase...
in the plane normal to the direction of propagation by a factor \( e^{i\theta} \), where \( \theta \) is the polar angle in the plane, effectively shaping the wavefront into a spiral, so that destructive interferences occur at the beam center. The nodal pump is below referred to as vortex. For simplicity, the anti-nodal pump is defined as a Gaussian, concentric with the vortex pump as well as with the Gaussian probe.

![Diagram](image)

Fig. 1. (a) Illustration of the quantized IR absorption in a two-level system (left), of the saturation where the excited population is at maximum (middle), and of the pump-probe sequence where the population is saturated by a first pulse and probed by a second delayed pulse (right). (b) Description of the differential pumping scheme where the VD-IR PSF is created by alternating nodal and anti-nodal pump profiles.

3. Formalism

To study the feasibility of the proposed concept and predict realistic PSF, VD-IR images of a CH\(_2\) stretch vibration at ca. 3.5 \( \mu \)m in octadecyltrichlorosilane were simulated, taking into account of empirical values of the absorbance and relaxation time of the CH stretch modes [45–55]. The change in intensity due to stimulated absorption/emission [56], along the coordinate \( z \) defined as the axis of propagation of the light pulses, is readily inferred from

\[
\frac{dI}{dz} = \frac{hc}{\lambda} k(\mathbf{r}) \rho(\mathbf{r}) \Delta N(\mathbf{r}, t) I(\mathbf{r}, t),
\]

(1)

where \( k \) defines the stimulated emission/absorption coefficient, \( \rho \) is the density of oscillators, \( \Delta N \) is the difference in relative population density between the levels \(|1\rangle\) and \(|0\rangle\), \( I(\mathbf{r}, t) \) is the local intensity at a given time \( t \) and sample position \( \mathbf{r} = (x, y, z) \), with \((x, y)\) the coordinates in the sample plane set normal to the direction of propagation of the light. \( h \) and \( c \) are the Planck constant and the speed of light in vacuum. \( \Delta N \) is determined by

\[
\frac{dN}{dt} = -\Gamma(\mathbf{r}) N(\mathbf{r}) - k(\mathbf{r}) \Delta N(\mathbf{r}, t) I(\mathbf{r}, t),
\]

(2)

where \( \Gamma \) is the deexcitation rate of \(|1\rangle\), and \( N \) the relative population density of the oscillators in the excited state \(|1\rangle\) at a given time and position in the sample.

The sample was modeled in space as an ensemble of voxels each containing a collection of independent oscillators, and a code was written in Matlab to solve the system of equations. The latter was developed using the backward Euler approach and independently for each set of \((x, y)\) coordinates. The solution is readily found by iteration with boundary conditions \( N(\mathbf{r}, t_0) = 0 \), where \( t_0 \) is a reference time before irradiation of the sample, and \( I(x, y, z_0, t) \)
describing the temporal evolution of the intensity impinging the sample at \( z_0 \) for the co-
propagative pump and probe pulses.

The departing probe pulse energy \( \Sigma \) was computed by integrating the intensity in the
sample plane \((x, y)\) and over time, at the coordinate \( z \) exceeding the sample thickness. IR
absorption images and sub-diffraction VD-IR images were computed by repeating the
calculation whilst varying the relative position of the sample with respect to the pulses. The
IR absorption and VD-IR signals are defined by

\[
IR(\%) = \left( \frac{\Sigma(z_0) - \Sigma}{\Sigma(z_0)} \right) \times 100, \tag{3}
\]

and by \( VD - IR \) (\% ) \( = \left( \frac{\Sigma_{\text{Gauss}} - \Sigma_{\text{vortex}}}{\Sigma_{\text{Gauss}}} \right) \times 100, \tag{4} \)

where \( \Sigma(z_0) \) is the probe pulse energy incident on the sample, and \( \Sigma_{\text{Gauss}} \) and \( \Sigma_{\text{vortex}} \) are the
probe pulse energy transmitted through the sample following a Gaussian or a vortex pump
pulse, respectively. The calculation neglects scattering and diffraction in the sample, which is
justified since its thickness is of a few microns maximum in transmission IRAS microscopy
and thus typically shorter than the wavelength, and since the whole transmitted intensity is
integrated over the sample plane.

In the focal plane, the spatio-temporal intensity profile of the Gaussian pulses is of circular
symmetry and defined by

\[
h_{\text{Gauss}}(r, t) = h_{\text{Gauss}}^0 \ e^{-r^2/w_0^2} \ e^{-(t-\Delta t)^2/\tau_0^2}, \tag{5}
\]

where \( r \) is the radial coordinate in the plane normal to the direction of propagation, and
where the full width at half maximum (fwhm) of the Gaussian is defined by \( 2\sqrt{\ln(2)} \ w_0 \), with
\( w_0 \) the Gaussian waist, and the temporal pulse duration by \( 2\sqrt{\ln(2)} \ \tau_0 \). \( h_{\text{Gauss}}^0 \) is a constant
adjusted to reproduce pulse energies ranging from 1.0 nJ to 1.0 \( \mu \)J for the Gaussian pump
pulses and 0.1 nJ for the probe. \( \Delta t \) is zero for the pump pulses and set to a finite value for the
probe, marking the pump probe delay. The vortex pulse intensity in the focal plane is written
\[ h_{\text{vortex}}(r, t) = h_{\text{vortex}}^0 \ r^2 \ e^{-r^2/w_0^2} \ e^{-(t-\Delta t)^2/\tau_0^2}, \tag{6} \]

with \( h_{\text{vortex}}^0 \) a constant adjusted to the desired vortex pump energy.

4. Empirical considerations

Aiming to a realistic prediction of the VD-IR microscopy performances, the fwhm of the
Gaussian pulses were adjusted to those expected with objectives of NA 0.7 and 0.85. The first
value is chosen because it is the largest NA reported for reflective objective, such as those
used in synchrotron IR absorption microscopy [10,58], and the second value because
aspherical lenses at 3.5 \( \mu \)m with a NA of 0.85 are commercially available. At the diffraction
limit, experiments have verified that an objective with NA 0.7 focuses a Gaussian beam with
\( \lambda \) at 3.5 \( \mu \)m into a spot of fwhm ca. 2.3 \( \mu \)m [10]. A fwhm of ca. 1.9 \( \mu \)m is thus readily
expected for a NA of 0.85. The beam waists \( w_0 \) are adjusted to reproduce these fwhms for
both the Gaussian pulses and the same waists are used for the vortex pulses.

Pump-probe spectroscopy of vibrational modes is best achieved with picosecond-long
pulses [55], affording suitable time resolution without compromising too much on the spectral
resolution, and a duration of 1.0 ps was then chosen here for all pulses. The spatial
distribution of the intensity of Gaussian and vortex pulses are shown in Fig. 2(a), along with their radial profile computed for an energy of 1.0 nJ.

![Image](image_url)

Fig. 2. (a) Spatial distribution (scale bar: 2.0 μm) of the intensity of Gaussian and vortex pulses computed for an energy of 1.0 nJ, a duration of 1.0 ps, and a waist of 1.6 μm (Gaussian fwhm: 1.9 μm), along with their corresponding radial profile. (b) Schematic of an octadecyltrichlorosilane layer on a dielectric IR transparent substrate. (c) Sketch of the optical path for the Gaussian probe and the alternating Gaussian and vortex pumps. BS, SPL, L, TG, D stand for beam splitter, sample, lens, time-gate, and detector.

The sample emulates a thin film of octadecyltrichlorosilane self-assembled on a fully transparent dielectric substrate (see Fig. 2(b)), for which experimental measurements reveals ca. 0.5% of absorption by the CH₂ stretch modes in homogeneous films [45]. This value is reproduced with Eq. (1) and Eq. (2) for $k$ equals 1.4 nm²$f{J}^{-1}$ and $\rho$ 31.5 nm⁻³, accounting for a uniform thickness of 2.0 nm confined into a single pixel, an area of 27 Å² per molecule, and 17 CH₂ moieties per molecule.

With experimental observations of the lifetime of CH stretch modes revealing values spanning from a little as a few picoseconds up to several hundreds of picoseconds [46–55], the decay rate $\Gamma$ was set at 0.1 ps⁻¹.

A sketch of the optical path for the Gaussian probe and the alternating Gaussian and vortex pumps is presented in Fig. 2(c). The pump pulses are focused on the octadecyltrichlorosilane sample and followed by the delayed Gaussian probe pulse which is separated after transmission through the sample by a time-gate and eventually integrated by a detector. In line with Eq. (4), the VD-IR signal is built as the difference between the transmitted probe pulse energies at the detector with Gaussian and vortex pumps. Images are generated by scanning the sample in the beam paths.

The intensity at which the saturation of $N$ occurs has been computed by observing the evolution of the population of $|1\rangle$ in a single pixel as a function of the intensity of a pulse of duration 1.0 ps (see Fig. 3). The data reveals a saturation threshold of ca. $1.1 \times 10^2$ kW.μm⁻² for the emulated sample which is consistent with earlier observations [55]. From Fig. 2(a) and Eq. (6), it is seen that energies above 10 nJ are required to achieve saturation with the vortex pump. These energies and intensities are readily available in the mid-IR with tunable picosecond sources based on non-linear optical conversion and pumped by pulsed lasers.
Fig. 3. Local evolution of the population $N$ as a function of the intensity. A saturation threshold of $1.1 \times 10^2$ kW$\mu$m$^{-2}$ is determined. (inset) Evolution of $N$ with time at the intensity peak of a Gaussian pulse centered at 0 ps with energy of 1 nJ (continuous line), 10 nJ (shortest dash), 0.10 $\mu$J (long dash), and 1.0 $\mu$J (longest dash).

5. Point-spread functions

The PSF for the IR absorption and VD-IR microscopies have been simulated by computing the IR(%) and VD$-$IR(%) images for a sample where the molecules are found in a single pixel of dimension $25 \times 25$ nm$^2$, and where all others pixels are left transparent ($i.e., k$, $\Gamma$, and $\rho$ are all null). Since pumps and probe are here always of circular symmetry, only plots of the radial evolution of the PSF are shown. The Gaussian and vortex pumps were set to the same energy and waist, and varied from 1.0 nJ to 1.0 $\mu$J, and the Gaussian probe at 0.1 nJ. The PSF are shown in Fig. 4(a) for a NA of 0.85 and a pump-probe delay of 2.0 ps.

The PSF for the diffraction limited IR absorption image is shown as a reference and reproduces the expected fwhm of 1.9 $\mu$m, commensurate with the dimension of the Gaussian probe in the sample plane. From the concept illustrated in Fig. 1(b), the VD-IR PSF is expected to be narrower and this is confirmed by the simulated data presented in Fig. 4(a) and Fig. 4(b). With the pumps at 1.0 nJ, thus about an order of magnitude below the saturation threshold, the VD-IR PSF is already reduced down to 1.05 $\mu$m. This value is about half the radial distance between two intensity maxima in the vortex pattern (2.2 $\mu$m) and is below-the-diffraction-limit (1.9 $\mu$m). The improvement in resolution is reminiscent of structured illumination microscopy that exploits sinusoidal illumination patterns to double the spatial resolution in wide-field microscopy [59]. Increasing the pump energy to values that are sufficient for the intensity to exceed locally the saturation threshold leads to a further improvement in resolution with a fwhm of 250 nm at 0.1 $\mu$J ($i.e.,$ for a maximum vortex intensity ca. 8 times above the saturation threshold), and below 100 nm for higher energies. Figure 4(b) highlights the influence of the dimensions of the vortex profile. Replacing the objective by one of NA 0.7 increases the diameter of the ring of maximum intensity from 2.2 $\mu$m to 2.85 $\mu$m, and the PSF fwhm is scaled up accordingly, with for example a value of 390 nm at 0.1 $\mu$J. The value also reflects the decrease in local intensity at same pulse energy due to the increase in irradiated area.

Although the PSF for VD-IR microscopy exhibits mainly a point-like geometry, negative contributions at large radial displacements are seen when the excited population $N$ in the outer rim of the probed area differs between vortex and Gaussian pumps (see Fig. 4(a) at 1.0 nJ). However, these negative contributions vanish for large pump energy when the entire probed area is effectively saturated for both pumps.

The magnitude of the VD-IR signal when the pixel of molecules coincides with the center of the beams is presented in Fig. 4(c) for different pump-probe delay $\Delta t$, and for different
pulse energies, with NA at 0.85. For short delays such as 2.0 ps and energies close or above the saturation threshold, the VD-IR signal corresponds to the same percentage of intensity measured at the detector as for the IR absorption microscopy. Figure 4(c) illustrates the exponential decrease of the VD-IR signal when the pump-probe delay $\Delta t$ is increased. The decay occurs with a rate equivalent to the decay rate $\Gamma$ of the excited oscillators (i.e., 0.1 ps$^{-1}$), which is understandable given that at longer delays the probe interrogates a population that is exponentially decreased (see inset in Fig. 3).

![Figure 4](image)

**Fig. 4.** (a) PSF for VD-IR microscopy for pump energies of 1 nJ (long dash), 10 nJ (short dash), 0.10 $\mu$J (shortest dash), and 1.0 $\mu$J (continuous line). The IR absorption microscopy PSF is also shown (longest dash) for comparison. (b) Fwhm as a function of vortex pump peak intensity for NA = 0.7 and $\Delta t = 2.0$ ps (square), for NA = 0.85 and $\Delta t = 2.0, 6.0, 9.0$, and 15 ps (circle, triangle up, triangle down, and diamond). The data are computed for energies 1 nJ, 10 nJ, 0.10 $\mu$J, and 1.0 $\mu$J. The horizontal dotted line marks the fwhm for the IR absorption image. (c) VD-IR maximum signal as a function of $\Delta t$, for energies of 1 nJ (triangle down), 10 nJ (triangle up), 0.10 $\mu$J (circle), and 1.0 $\mu$J (square). The horizontal dotted line marks the maximum signal for the IR absorption image.

IR and VD-IR images at 3.5 $\mu$m have been simulated with the pump set at 0.10 $\mu$J for an array of nine domains of octadecyltrichlorosilane ($200 \times 200$ nm$^2$). As shown in Fig. 5, the pattern is clearly resolved and the VD-IR signal is then ca. $0.4 \times 10^{-2}\%$ of the total energy fluency at the detector. The signal-to-noise ratio required for measuring a molecular domain of $200 \times 200$ nm$^2$ remains thus of the order of $10^{-7}-10^{-6}$ (i.e., for a VD-IR magnitude a decade above noise level), a value that remains acceptable in view of the noise-equivalent power in modern IR detectors (e.g., 0.2 pW Hz$^{-1/2}$ for mercury cadmium telluride) [20]. Indeed, using such a detector in conjunction with lock-in amplification, Fourier-transform thermal-source IR SNOM imaging has been demonstrated with a spatial resolution below 100 nm and an integration time of 40 ms per pixel, in a configuration where the scattered power was only $10^{-8}$ that of the power in the reference arm [20]. A comparison between Fig. 5(d) and Fig. 4(a) shows that the VD-IR signal scales linearly with the molecular domain dimensions, with maximum contrasts of ca. $0.4 \times 10^{-2}\%$ and $0.7 \times 10^{-4}\%$ for domains of $200 \times 200$ nm$^2$ and $25 \times$
25 nm^2, respectively. Figure 4(a) also shows that for a given molecular domain the signal contrast for VD-IR and IR microscopy is the same at short pump-probe delays and that both methods have the same detection capability. Thus, although the instrumental noise (i.e., laser source, time-gate, detector, etc) will ultimately limit the sensibility of VD-IR, the signal contrasts are found sufficient in the sub-micron and nanoscale regimes.

6. Damage threshold

Akin to the RESOLFT techniques affording nanoscale resolution in fluorescence microscopy [24–30] the resolution for VD-IR is theoretically bound only by the pump energy, and the latter can be, practically, increased up to the damage threshold of the sample. A brief survey of past IR pump-probe experiments of CH stretch modes in organic materials and of the damage threshold in inorganic materials is thus useful to assess the potential performance of VD-IR. Because pulsed lasers are necessary to achieve peak intensities above the saturation threshold, the averaged power that will be received by the sample is relatively moderate. Assuming a standard repetition rate of 1.0 kHz, the power here is at most 1.0 mW, since 1.0 μJ is the highest pulse energy considered in this study. This is a relatively modest power and similar to the values used in near-field probe imaging of polymer beads with a laser power of ca. 1 mW focused within a 10 μm beam spot [22]. The power values are also similar to those used in synchrotron IRAS microscopy of samples including biological cells where the broadband beam has been focused without damage on the same areas on samples [60,61].

Bearing in mind the typical temporal structure of a synchrotron source [11], the much shorter pulses required for VD-IR imply higher peak intensities and their values with respect to the damage thresholds in organic and inorganic materials must be considered. The average energy density of the vortex pump at the sample is 5 nJ/μm^2 for a pulse of 0.10 μJ that is focused on an area of radius ca. 2.5 μm (see Fig. 2(a), NA = 0.85) and for which a resolution of about λ/14 is calculated (see Fig. 4). The energy density expected for 0.10 μJ is thus about an order of magnitude below the damage threshold of dielectric substrates such as CaF$_2$ and silicate exposed to ps IR pulses [62,63]. Moreover, sum-frequency generation (SFG) images of the CH stretch modes in organic monolayers [64] and IR pump-SFG probe spectra of CH stretch mode on diamond [48] have been successfully acquired with an energy density of ca. 10 nJ/μm^2, suggesting that the damage threshold is close to or above that value. It seems thus reasonable to assume that VD-IR affords spatial resolutions better than 250 nm (λ/14) for these materials and that a resolution below 100 nm is also possible (see Fig. 4(b)).
For molecules in a solution or at the interface with another liquid, a brief survey of IR pump-probe spectroscopy investigations reveals that saturation of CH in organic molecules is typically made with pulse energy densities of the order of several nJ.μm⁻² [52,53,65]. For VD-IR microscopy, the pump energy will thus be preferably limited to values ca. 0.10 μJ resulting in a spatial resolution of the order of 250 nm or higher. For IR nanosecond pulses, the ablation threshold of soft tissue has been estimated at ca. 20 nJ.μm⁻² [66] and ablation experiment supports the negligible heating of tissue at low repetition rates [67], with complete cooling between pulses.

7. Conclusions

A conceptual framework has been proposed for imaging the IR absorbance of samples at sub-diffraction spatial resolutions and simulation of the associated PSF has been discussed. The pulse energies required to pattern the vibrational depletion to achieve imaging beyond the diffraction limit have been determined on the basis of empirical parameters. It has been found that, for energy density of several nJ.μm⁻² i.e., roughly 10 times above the saturation threshold, the fwhm of the VD-IR PSF can be as low as λ/14, which highlights the possibility of IRAS imaging beyond the diffraction limit. For monochromatic IR pulses, these energy densities are estimated to be below the damage threshold for thin organic films on dielectric or metallic substrate, and close to for soft tissue and organic molecules at soft interfaces. Chemical imaging based on the measure of the IR absorption by vibrational modes in the far-field is thus possible at sub-micron resolution and down to the nanoscale.

Acknowledgments

The authors acknowledge Dr. Ian Clancy from the University of Limerick for his support with the simulations. C.S. acknowledges funding from the Integrated Nanoscience Platform for Ireland (INSPIRE), initiated by the Higher Education Authority in Ireland within the PRTLI4 framework. N.H. acknowledges financial support from the CERUNA-FUNDP foundation. A.P. is a Research Director of the Belgian Fund for Scientific Research (FNRS-FRS). The LANIR research leading to these results has received funding from the European Community’s Seventh Framework Programme (FP7/2012-2015) under grant agreement n°280804. This communication reflects the views only of the authors, and the Commission cannot be held responsible for any use which may be made of the information contained therein.