Diffracted Light Contrast: A chemical application

by

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ABSTRACT

There is a tremendous interest in the development of microscopy tools that can provide important information at a cost significantly smaller than those offered by traditional electron microscopes. With the purpose of learning about a new microscopy tool and at the same time contribute to the current body of understanding related to the chemistry of explosives, we report here on spectroscopy and microscopy measurements performed on trinitrotoluene (TNT). Crystals and non-crystalline TNT samples were characterized with diffracted light microscopy (DLC), near IR spectroscopy and UV visible absorption spectroscopy. DLC is an optical microscopy tool under development elsewhere claimed to allow 3 dimensional observations of materials. The technique was employed in the characterization of crystalline and non crystalline TNT particles, exposed and non-exposed to sunlight. A colored solution is observed upon sunlight exposure to TNT in the presence of a protic solvent. The solution exhibit light absorption in the visible region of the spectra: no absorption is observed in TNT solutions not exposed to sunlight. New bands are observed in the near IR spectra of TNT deposits exposed to sunlight. The advantages and limitations of the DLC to the characterization of this system as well as the chemistry of sunlight exposed TNT based on our results will be discussed.

RESUMEN

Existe un gran interés en el poder desarrollar herramientas en el área de la microscopia que provean información importante a un costo significativo, el cual ofrecen microscopios tradicionales, a diferencia de tecnología avanzada como lo es la microscopia electrónica. Con el propósito de aprender sobre una nueva herramienta de microscopia tradicional y al mismo tiempo contribuir al conocimiento que se tiene sobre la química explosivos, reportamos medidas espectroscópicas y microscópicas desarrolladas en 2,4,6 trinitrotolueno (TNT). Muestras de TNT cristalino y no cristalino fueron caracterizadas por microscopia de difracción de luz (DLC), espectroscopia de infrarrojo cercano (NIR) y espectroscopia de absorción ultravioleta visible. DLC es una técnica de microscopia tradicional en desarrollo, que permite el obtener imágenes de materiales en tres dimensiones. La técnica fue aplicada para la caracterización de partícula de TNT cristalina y no cristalina, expuesto a radiación solar y no expuesta a radiación solar. Una solución coloreada se observa luego de exponer las muestras a radiación solar en presencia de un solvente prótico. La solución exhibe absorción de luz en la región de luz visible del espectro: no se observa absorción de luz en solución no expuesta a luz solar. Nuevas bandas se observan en el espectro de IR cercano de depósitos de TNT expuestos a la luz solar. Las ventajas y desventajas de la técnica de DLC en la caracterización de este sistema, como la química envuelta en el TNT expuesto a luz solar, basada en nuestros resultados, serán discutidas más adelante.

Dedication of this work belongs to my little treasure, Janmarcos, for being my inspiration… To my dad, Crescencio, who can't be part of this goal, but I know his so proud of me, now in God's presence… I miss you… To my parents Tago and Paquito for being extremely supportive, for never leave me alone, always have faith in me; for give me the strength and inspiration to go on and overcome every stone in my way. Without them I would never be able to be what I am today.

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CHAPTER 1

1.1 DIFFRACTION LIGHT CONTRAST

The function of any microscope is to enhance resolution**.** The microscope is used to create an enlarged view of an object such that we can observe details not possible with the human eye. Diffracted light contrast (DLC) is a new microscopy technique recently developed. The discovery of the technique emerged from the diffracted light of a convex edge plate, which can be used to form high quality; shadowcast image on any light microscope.[1] When using a convex edge to generate the diffracted light, each point along the edges will serve as a point of diffracted light. The discovery of a simple technique to improve contrast enhancement and resolution has an important role in experimental chemistry as well as in the biological field.

What is diffraction and how it works? Diffraction is the spreading out of light from its defined path. When diffraction occurs, typically appears as dark and bright fringes. This is a consequence of an advancing wavefront blocked by an object o apperture. The apertures can be a slit, circle shape or rectangle shape where the rays can pass through. In Diffracted Light Contrast the rays of light are blocked by an edge plate. The direction of the rays is consider to be collimated while the rays collide with the surface of the edge plate.

When we talk about collimated light, refers to these rays that are in a parallel direction and will spread slowly as it propagates along the space. The edge plate serves to the responsible tools to created hose ray and it an be called as a collimator. The collimator has the role of aligned the beam of light (as particles or waves) that comes from all directions. In optics, a collimators can consists of a curved mirror or lens with a light source and an image in focus. In DLC the collimator is represented as an opaque edge plate that help the rays to be collimated through the optical pass while it collide with the edge of the interference. What this mean is that the rays doesn’t disperse with distance or that will disperse minimally.

The figure 1.1 represents an schematic mechanisms of how rays spread in all direction, travel throght the path way between the source of light and collide with the interference upon the bright field. The collide rays are collimated through the specimen at the stage. Those rays pass through the specimen and though the optical lens that are collected to created am image of the specimen that is under study.

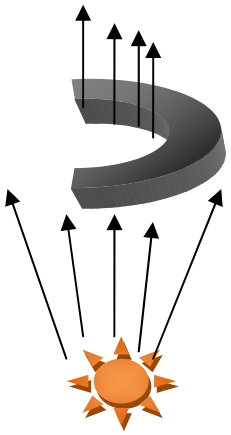


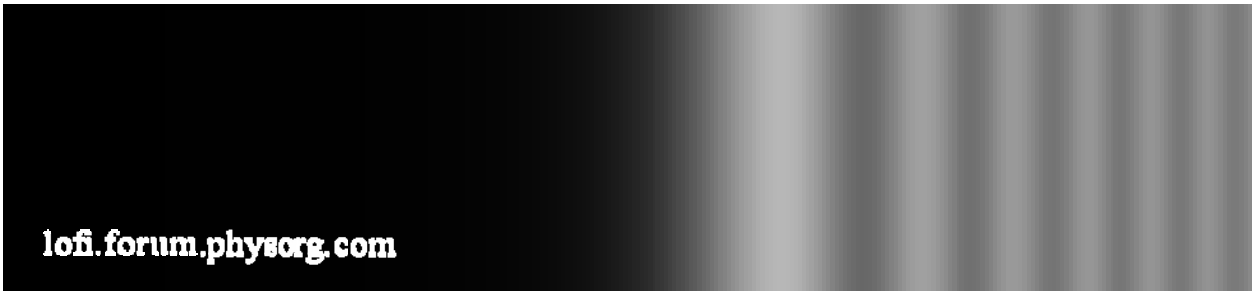
Figure 1.1: Schematic representation of bend rays through the edge plate. Rays collimated when its pass through the edge of the interference.

In microscopy the source of light is typically a Tungsten electrical bulb of 6 to 12 Volts and it is regulated by the condenser. The function of a condenser is manipulated or alters the quantity of light that travels through the optical path. In DLC the condenser and diaphragm has to be completely opened such that the maximum rays of light could pass through the collimator without any manipulation. In microscopy this is called as a Kohler illumination that maximizes the brightness and the uniformity of the illuminated field

When rays are near the collimator\(edge plate), rays are diffracted by the edge of the interference and generates a diffraction pattern. This diffraction can be described as the propagation of finite waves in a free space. There are different diffraction patterns that described the light behavior when it collides with a slit, a circle shaped, rectangle shaped or an object that interrupt the rays spreading. We can refer as Frauhofer diffractios as a fundamental explanation of how its work in DLC.

When light collide with the diffracting apertures or the interference, the rays must be parallel or close to parallel, as it reach the diffracted object. The point in which the diffraction is observed becomes the Fraunhofer plane.

The focal plane of a lens is therefore effectively formation of Fraunhofer diffraction. Fraunhofer diffraction occurs when both incident and diffracted wave are considered to be in plane. We can refer to the Huygens principle that underlies the idea that each point on a wavefront acts as a source of secondary wavelets. This means that when you have a wave, you can view the edge of the wave creating circular waves. These waves can be combining together in most case to continue propagation. How the pattern diffraction does is generating through the image plane? Joseph van Fraunhofer found that when light pass through a lens, it produce a diffraction pattern. What he observed was a fringed shadow who called diffraction pattern. The diffraction pattern will depend of the size and shaped of the aperture or interference in used. Figure 1.2 represents a Fraunhofer pattern viewing of what is produce when light is affecting by this phenomena.



DLC allows shadowcast imaging to be performed on student laboratory microscope. A convex edge generated a high contrast, a three dimensional image appearance with high resolution. The shape of the edge plate increases the contrast of an image in an optical microscope. The light rays bend around the edge and generates new wave fronts at sharp edges.

1.2 INTRODUCTION

Because of the enlargement, resolution is often confused with magnification, which refers to the size of an image. In general, the greater the magnification, the greater the

resolution, but this is not always true. There are several practical limitations of lens design which can result in increased magnification without increased resolution. The value for resolution may be determined in one of two ways. It can be measured as the smallest distance between two points, which allows us to see the points as distinct. With this measurement, resolution increases as the distance decreases. For instance, there is an inverse correlation between the limit of resolution and what you actually resolve. The resolution of a lens depends on physical properties and which contains the contribution of all physical properties.

Putting all of this to practical use, it is apparent that resolution can be increased in three ways. The easiest method is to increase the angle of light incidence, by altering the position and/or design of the sub stage condenser. Second, the refractive index can be maximized by using specially manufactured lenses, and by controlling the medium through which the light travels, i.e. using immersion oil with lenses designed for this purpose. The third method is to decrease the wavelength of light used. For practical purposes, the wavelength has a larger effect on resolution than either changes in the angle of incidence or the refractive index. For maximum resolution, all three properties must be optimized.

An illumination system that direct rays onto the specimen along the optical axis, is called as bright field. For routine bright field microscopy, it is more convenient to work in the visible light range, and the shortest wavelength of visible light is blue. Thus, even inexpensive microscopes have incorporated a blue filter into their design, which is often referred to as a daylight filter.

1.3 PREVIOUS WORK

The invention of a new and simple microscopy technique to perform very high quality image on any light microscope, produce a great interest to understand how it works. This innovative invention is most generally optics theory and elements, but particularly to illumination system within bright-field microscope. As everyone knows, the purpose of a microscope is to provide magnification of small things or samples that can’t be visible to the

naked eye. Light microscope has an optical bright field, which is the most important subject of this invention. The magnified images is created when light pass through the specimen or sample, within the optical bright field. An illumination system that directs the rays onto the specimen along the optical axis is called as a bright field microscopy system. The rays pass through the field that surrounds the specimen and enters to the objective aperture.

The sample must be transparent, translucent or a combination through which light can pass for viewing. The light generated by the source, such as sunlight and others artificial light, is typically gathered by a collector lens and concentrated by the condenser upon the stage of the microscope. Artificial light such as electrical light bulbs, commonly made of tungsten, are more commonly used because they are convenient and predictable. Tungsten is one of the most used materials in electrical bulb, from 6 to 12 volts.

1.4 MICROSCOPY THECNIQUE

There exist various adaptations and techniques that have been developed through time to enhance a bright field microscope. One frequent goal, developed from a new technique, is to improve detection and differentiation of features within the specimen. Some examples of those methods are the staining in biological specimens, contrast enhancement technique such as oblique angle, phase contrast, differential interference contrast, and others. The use of staining specimen created disadvantages in some specific studies. For example, it generated differences in permeability and/ or absorption of the stain and the specimen. Another disadvantage is that once stained, the specimen is not return to the state it was before. As a consequence, the sample may not be analyzed by others methods. There are different techniques that help to visualized the sample without destroy it or alter the medium or natural environment.

1.4.1 Oblique Angles Technique

Contrasting the staining, there are other methods non destructive to the sample that do not alter the natural ambient of the specimen. The oblique angles technique, for example, produces reflection and refraction at the interfaces between materials, having small difference in index of refraction. This technique requires an eccentric mount in association with the condenser aperture. Using an eccentric mount, the aperture diaphragm may shift from a central position, which passes an equal amount of light in all directions about the central axis, to an off axis position which only passes illumination from one side of the central optical axis through the condenser to the stage. This technique results in a shadowcast image with improved contrast. With oblique angles technique, resolution of smaller features is sacrificed, but the depth of field increase due to reduce numerical apertures of the condenser. The depth of field represents the distance which is in focus along the axis of light transmission to the sample. In very microscopic samples, the depth of field is insignificant.

1.4.2 Oblique Light Diaphragm

Oblique light diaphragm provides an provides an independently slidable leaves and cam for moving the leaves and cam simultaneously. This technique illustrates a concave shape oblique light diaphragm which is mounted nearby the iris diaphragm. The oblique diaphragm includes a piece called leaf that blocks the light partially from one side. The concave shaped were illustrated by Diggins and Ott patent which as a result, reduces the numerical apertures of the condenser, but the depth of field increased. The purpose of this invention was to provide an oblique light diaphragm, simple and inexpensive to manufacture. The purpose of a diaphragm is to controls the amount of light passing through the opening stage and adjusts to control the amount of light coming through the light source. Then the rest of light pass through the condenser, which focuses the light passing through the

specimen. The technique of oblique diaphragm provided the simultaneously moved of the diaphragm and unconnected leaves across a light beam.

1.4.3 Microscope with variation of light

The invention relates to microscope that provide a light source to arrange to direct light onto a movable mirror that may be oriented so that at least a portion of light is reflected in the direction of the specimen being viewed. The invention provided a microscope with an illumination system which includes a fixed mirror arranged with a light source below the specimen. It may be oriented along the axis of the light source in order to reflect light each to the fixed mirror or to the specimen to be viewed. One of a great advantage is the easier way to created because of the simple design and economical to manufacture.

As a general description of the invention, is compose of a microscope having an objective located directly above the viewing stage. A light source is constituted with a lamp and the central axis of the ray is reflected by a mirror as the light ray is then reflected by the expected mirror substantially vertically upwardly as the light ray and through the object to be view. Figure 1.2 represents a drawing explanation of what occurs with the ray light reflected through the microscopy passed. The mirror, number as 5 in the drawing, is mounted in a carrier which is rotatable and visual indications are provided by a dial, for indicating the amount of rotation from a fixed point in order read the angle at which reflected ray (9) is directed obliquely through a transparent specimen slide.

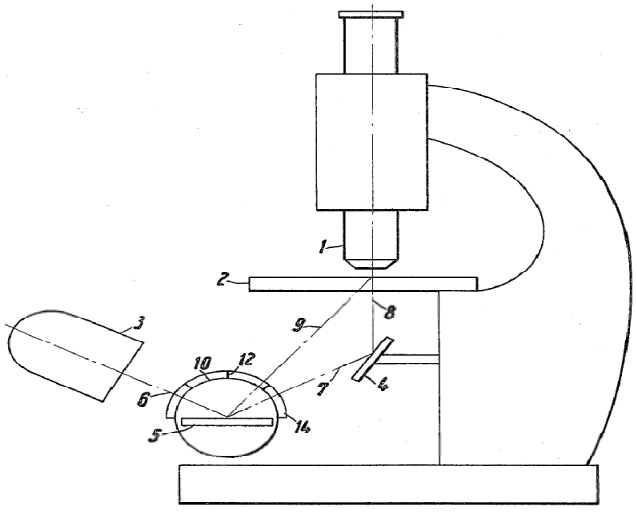


Figure1.3: Drawing representation of the light pass through the microscope as ray reflected by mirror.

1.5 SUMMARY

As a summary of the invention, there are three important outcomes. The first one is the combined device used for enhance the contrast of a refractive specimen. The device means the microscope having a stage for locating the specimen, the condenser, source of light within an optical path or field of light. The second is to generate a diffraction of the light that pass through the field, when interference or a convex edge plate is between the stage of the specimen and the condenser and the light source. The third outcome is the collection of this collimated rays that pass through the sample, that form a high quality image produce by a high contrast, high resolution and three dimensional effect.

CHAPTER 2

DLC Images Examples

2.1 DLC Example Experiment

The objective of this thesis is to contribute to the development of new microscopy tools with capabilities for 3 dimensional imaging. In this chapter we describe the experimental setup used for the DLC measurements and the approach employed in the calibration of the microscope for quantitative measurements. We take the opportunity to visit a few examples of the use of DLC for three-dimensional imaging.

2.1.1`Calibration of Microscope Camera for Quantitative Measurements

A scheme of the setup used to generate DLC image is illustrated on Figure 2.1 In a microscope equipped with standard optics the light used to obtain an image is not collimated. In DLC, the light is collimated by placing a curved interference on top of the light source. The interference at the edge of the curved interference results in a collimated light source and improved imaging capabilities.

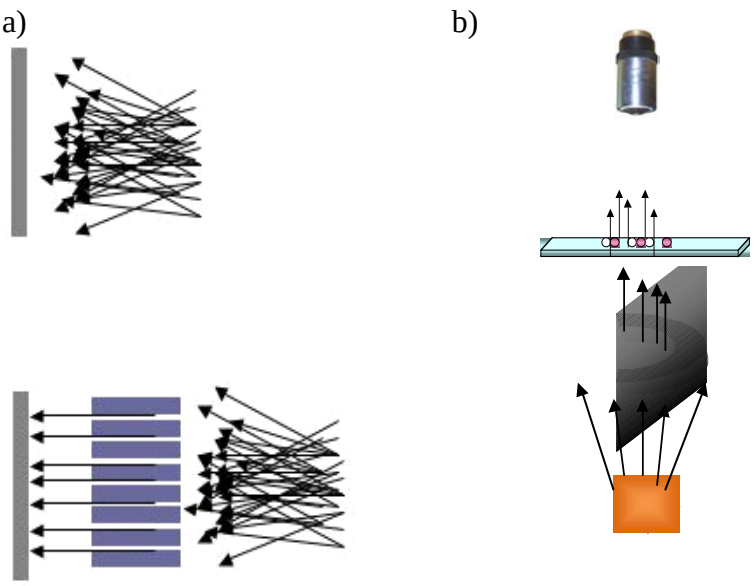


Figure 2.1: (a) Representation of rays dispersed vs. collimated rays. (b) Schematic representation of the experimental methodology.

A microscope ruler was employed to calibrate the microscope objective. The ruler is a scale with lines of known width and separation. Figure 2.2 illustrate images of a region of the ruler employed with a bench microscope using standard optics and with interference for DLC imaging. The images were obtained in the reflection mode with an Olympus LM Plan F1 objective and a magnification of 50 X. The white lines represent the scale while the darker region represents the space between the lines. The width and space among lines is indicated by a series of numbers, in microns, that appear at the bottom of the segment in the scale of the ruler. For example, 10-1 indicates lines that have a width of 10 microns and a line spacing of 1 micron. With the 10 X objectives we can easily resolve lines that are about 10 microns in width with a separation of 4 microns. The dimensions of the lines are used to calibrate the MOTIC software that is used to communicate between the PC and the camera. The same approach was employed to calibrate the software for the use of a 10 x and 100 x objectives. The same camera and software are used to collect the DLC microscope images discussed in the remaining of this thesis.

We wish to highlight that the use of a DLC microscope does not result in improved resolution. The ultimate resolution in a light microscope is diffraction limited by ($\lambda/2$). Assuming most of the visible light has a wavelength of about 400 to 500 nm, it is expected that the ultimate resolution to that can be attained ranges from 200 to 250 nm. The first advantage of the use of the DLC emerges from the natural reduction in light intensity that improves image contrast. The smaller amount of light reaching the camera when the interference is placed in the optical path of the DLC microscope reduces the brightness resulting in images with improved background. In addition, the collimation of the light reduces the scattering of the light produced at the edges of the object to be imaged, as highlighted by the arrows indicating the edges of the lines of the ruler. Collimated light is when rays are nearly parallel and will spread slowly as it propagates. The inserts in figure

2.2 highlights the 3d appearance of the DLC images as oppose to those obtained with the use of standard optics.

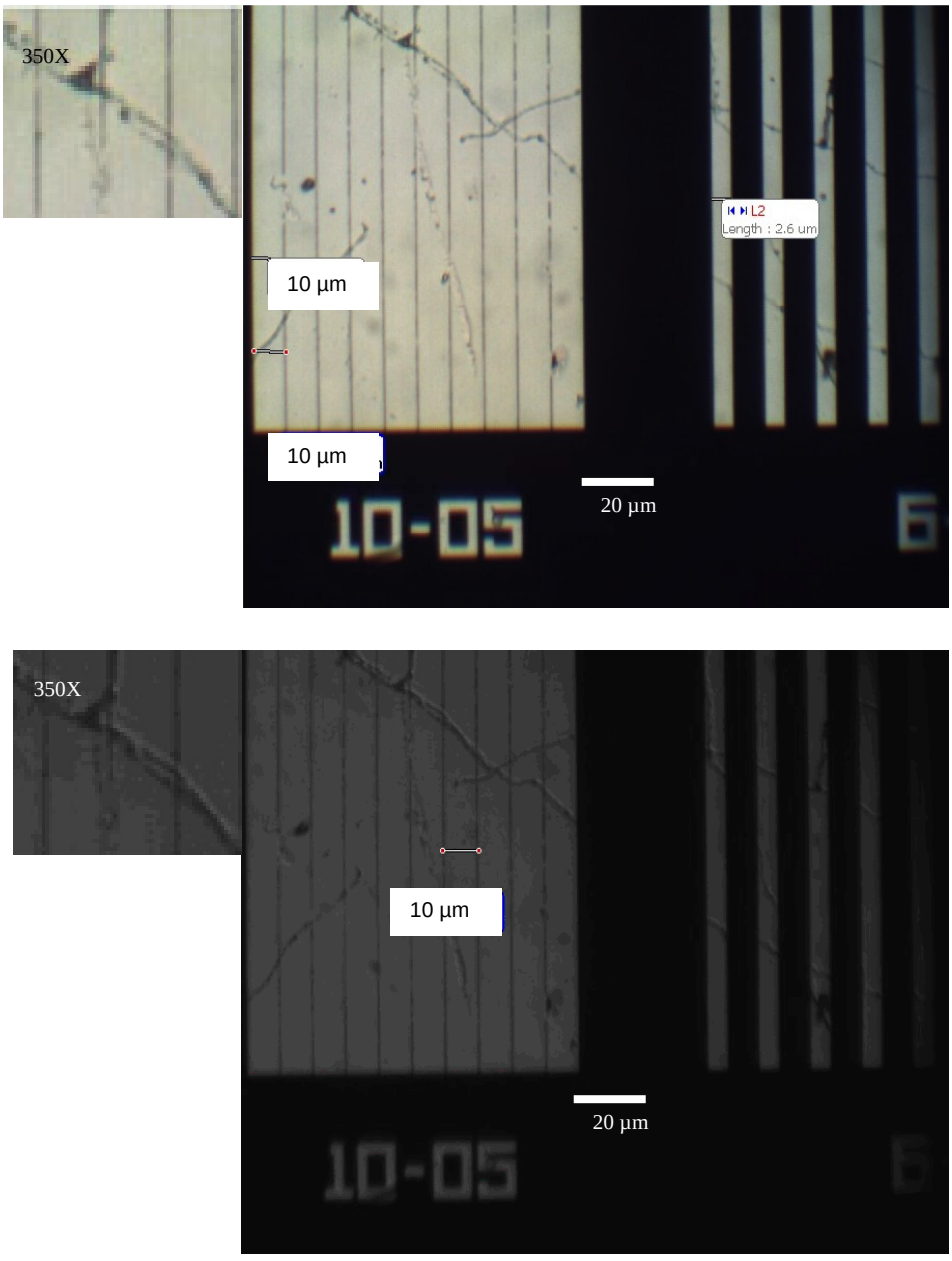


Figure 2.2: Microscope and camera calibration with a microscopic ruler with a 10 micron scale. A 50 x objective was employed for the measurements.

Measures: The whole capture squared is about l = 152 µm x a= 204 µm. The measures of the selected area is about l= 26 µm x a= 32 µm.

2.1.2: Interference width calibration

After all the calibration procedure, we calibrate the instrumentation in terms of distance and width of the interference. The purpose of this calibration is to confirm the specific distance and width of the interference that is require for the DLC technique. Figure 2.3 represents a width interference comparison in relation to the ray bending. If we use a thin interference, all rays will not bend in a parallel to the sample. With a width between 2 -3 mm, the effect of collimated light can be appreciated. An interference with a bigger width does not made a critical difference in the ray collimation, because rays keep travel in a parallel direction through the sample.

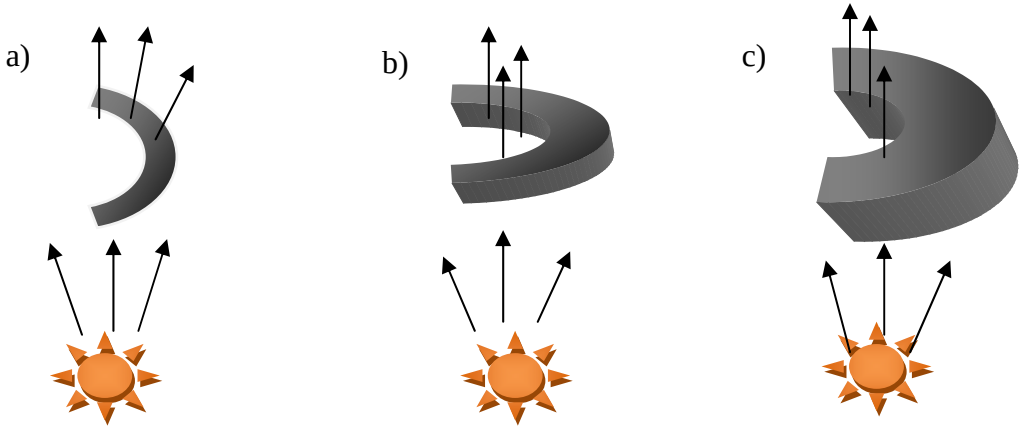


Figure 2.3: Schematic representation of width interference vs. ray bend.

The interference width that better acts as a collimator was between 2-3 mm. The measurements and imaging capture were doing using the same interference, with the same dimensions, at the same microscopy scale.

2.2 Gold nanoparticles

Figure 2.4 illustrates images obtained with standard microscope optics and with an interference used to generate DLC images of gold nanoparticles. The images were obtained with an objective magnification of 50 x. The insert at the right hand side of each figure represent a digital magnification (x 350 %) of the area highlighted by the squares in the original images. While it is difficult to observe clearly details associated with the gold nanoparticles it is possible to identify some 3d features that cannot be identified in images obtained with a microscope operated with standard optics.

Our digital camera software is equipped with a tool to allow relative estimates of the light intensity as a function of spatial position. The white inserts in figure 2.3 represents the dependence of the light intensity reaching the camera as a function of position in the image.In the case of the sample examined, we observe a light intensity fluctuation likely resulting from the absorption of light by the gold nanoparticles.

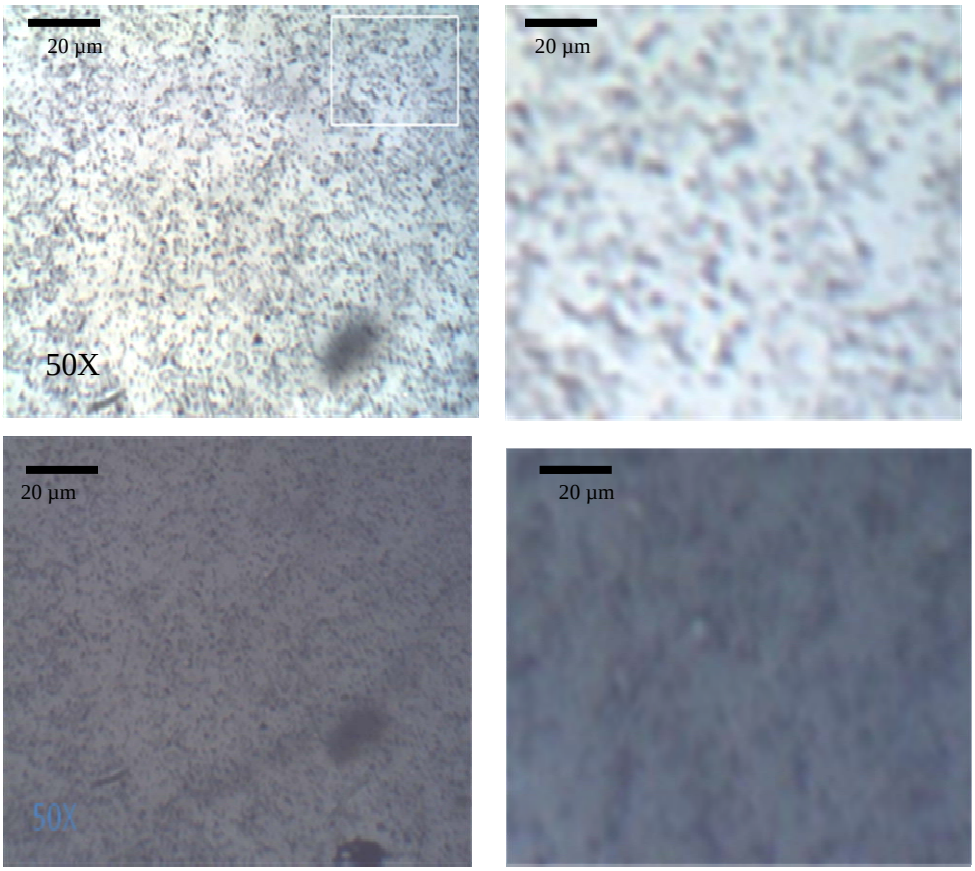


Figure 2.4: Images obtained with standard microscope optics and with an interference used to generate DLC images of gold nanoparticles

The effect of the shape of the interference was also studied. The left and right hand side of figure 2.5 illustrates DLC images of water drops obtained with concave and convex interference, respectively. In all our measurements, the use of a concave interference resulted in images with a well defined 3 dimensional structure.

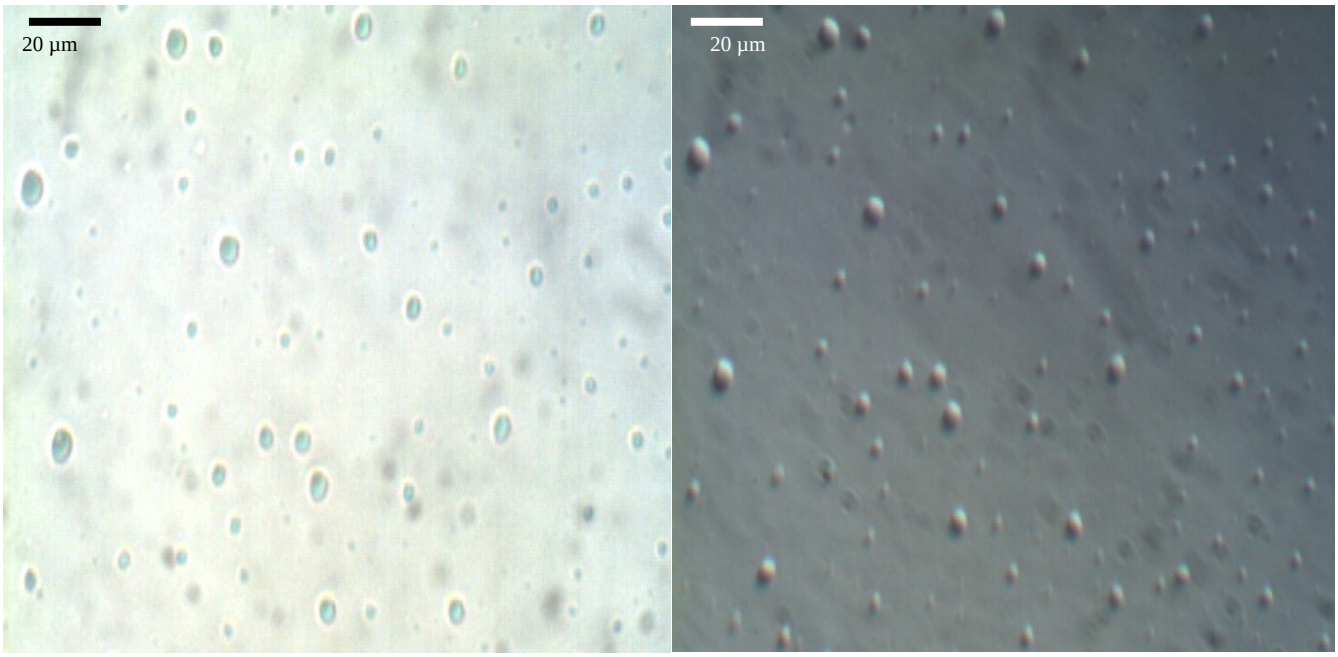


Figure 2.5: Images of drops solvent of gold solution with a convex (left) interference and concave interference (right).

2.3 TEM Gold nanoparticles images: Limit of detection

To establish the limits of detection of the DLC technique, we use the Transmission Electron Microscope (TEM). The transmission electron microscope (TEM) operates on the same basic principles as the light microscope but uses electrons instead of light. What you can see with a light microscope is limited by the wavelength of light. TEMs use electrons as "light source" and their much lower wavelength make it possible to get a resolution a thousand times better than with a light microscope.

One of our goals by using the TEM was to prove that the measures obtained with the light microscope were consistent with measures with the TEM. The measures obtained by the TEM were taking by a calibrated screen localized in the instrument. The scale is calibrated in centimeter. To calculate the real size of the sample we were measure, we have to use the measure in cm, obtained by the TEM scale and divide it by the magnification in use. For example, figure 2.6 shows an island of gold nanoparticles. First, we observed a particularity in the gold nanoparticles that forms holes between particles. The measure of the hole, with the TEM rule was about 1.5 cm. Now, we convert this value in meters, then, divided by the magnification, in this particular was 40000. Here is an example of the calculus:

Calculus: 1.5 x 10-2 m/ 40,000 = 3.75 x 10-7/ 1.0 x 10 –9 = **375 nm**

In figure 2.7 we measure the size of the whole island of gold nanoparticles. By TEM scale we obtained a measure of 975 nm. When we compare that value with the optical measures of nanogold clusters, figure 2.9, we can confirm that the optical measures are according to the real size of the particles. What we are seeing at the microscope are the agglomerates of gold that are between 500 to 1500 nm. We can conclude that DLC help us to resolve at nanometers scale.

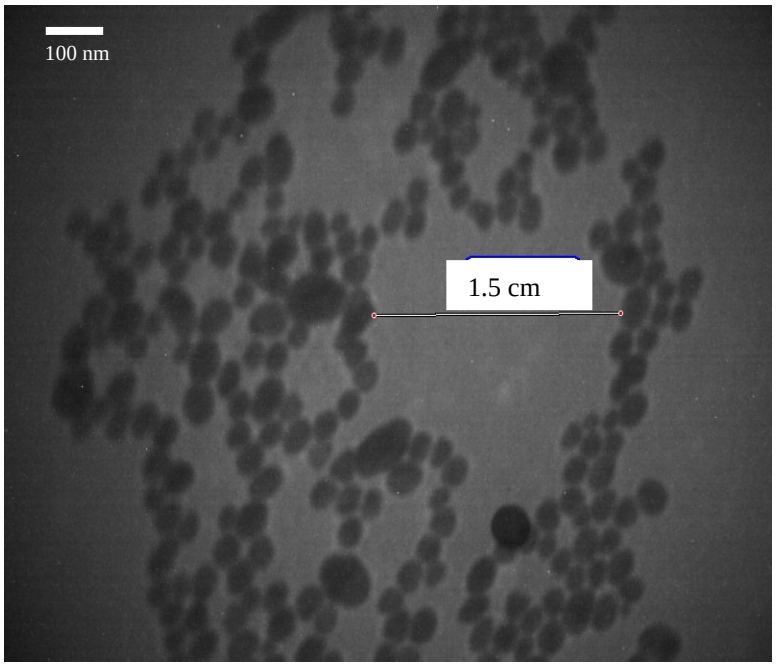


Figure 2.6: TEM images of gold colloid with 40K of magnification. Hole measure with TEM scale about 1.5 cm.

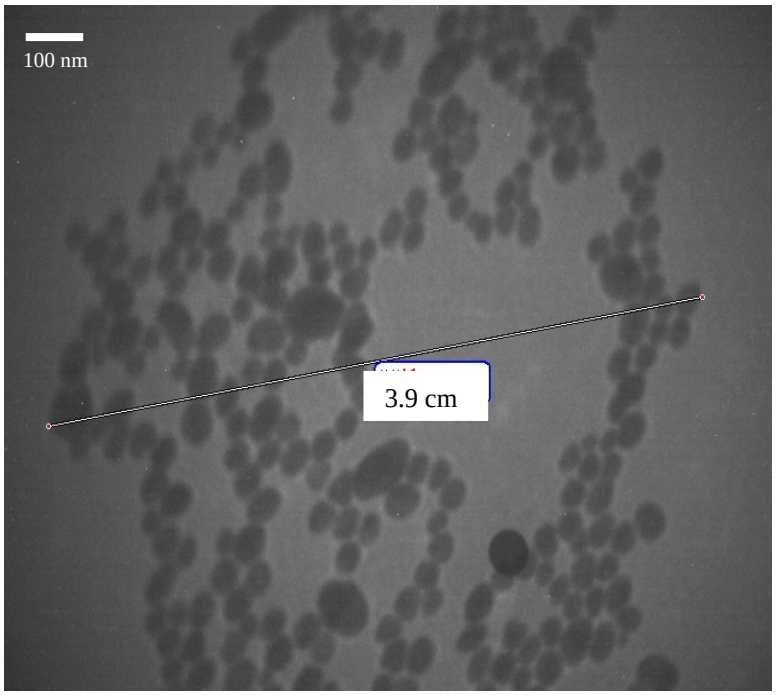


Figure 2.7: TEM images of the nanogold island. The whole measure is about 975 nm. (Calculations were explained in paragraph before)

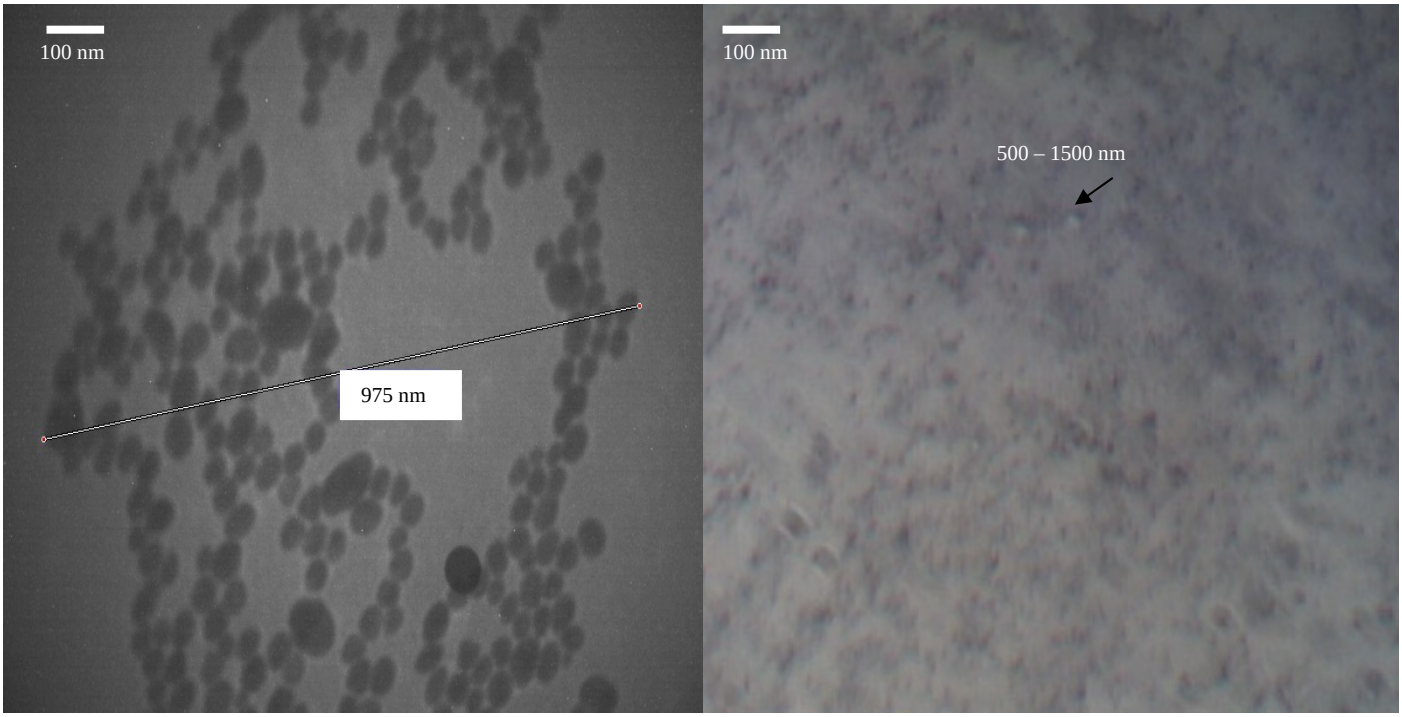


Figure 2.8: TEM (40000x) and DLC (50x) images comparison of nanogold particles.

We can compare at different magnification and with different instrumental techniques that it is possible resolved at a nanoscale using old instrumentation with a new microscopy technique.

If we observed the TEM image, we observed that the gold particles have a tendency to form agglomerates of the same size. By choosing an amount of same size nanogold particles, we took an average, which is about 30 nm.

CHAPTER 3

TNT: A Case of Study

3.1 Introduction

2,4,6-Trinitrotoluene (TNT) is a crystalline solid used as an explosive in military armaments and a chemical intermediate in the manufacture of dyestuffs and photographic chemicals (Sax and Lewis 1987). It is slightly soluble in water (104 to 113 mg/L) and soluble in tolune, acetonitrile, alcohol, ether, acetone, benzene and carbon disulfide. It has a density of 1.654 g/mL, a vapor pressure of 8.02×10^{-6} mm Hg at 25°C, and a partition coefficient (log K_{ow}) in water of 1.60 (EPA 1990). TNT is extensively metabolized by nitroreduction to various derivatives, including hydroxylamino-dinitrotoluenes, amino-dinitrotoluenes, and diamino-nitrotoluenes (El-hawari et al. 1981). The methyl group can also be oxidized to form benzyl alcohol and benzoic acid derivatives. Some metabolites may undergo conjugation reactions with glucuronic acid.

TNT is known to exist in crystalline and the somewhat less studied non crystalline forms. Differences in the vibrational spectra of these forms of TNT have been documented, presumably emerging from the smaller number of interactions that take place in the non crystalline form. A TNT crystal, on the other hand, has been subject to numerous vibrational studies. Differences in the morphology of these two TNT forms are intriguing. The non crystalline form is easily prepared upon drying TNT deposits prepared from diluted solutions while well defined TNT crystallites are easily obtained from concentrated TNT solutions.

3.1 Characterization of the crystalline and non crystalline forms of TNT

Near IR spectroscopy is a technique that relies on measurements of vibrational transitions. Traditionally, vibrational spectroscopy measurements are performed in the mid infrared. Interpretation of the mid IR spectra of molecules is typically made in the context of the harmonic oscillator approximation. In this approximation, transitions are allowed when the dipole moment associated with a mode changes. This imposition results in the so called infrared selection rule for an allowed transition from state n_i to the state n_f:

$$\Delta n = n_f - n_i = \pm 1$$

Where the plus and minus sign indicate that the transitions absorbs or releases energy. In general, the difference in energy between the levels of the oscillator is given by:

$$\Delta E = E_f - E_i = \Delta n \, hw$$

Where h and w are Planck's constant and the oscillator vibrational frequency, respectively. Transitions that do not follow the selection rule described by equation 3.1 are said to be forbidden. Although they are forbidden, there is always a finite probability to observe such transition. Near IR spectroscopy measures transitions for which $\Delta n = \pm 1$, that is measures forbidden transitions in the harmonic oscillator approximation. This transitions are called overtones, with the first overtone corresponding to $\Delta n=2$, the second overtone corresponding to $\Delta n = 3$, and so on. The intensity of allowed transitions in the mid IR makes it a little difficult to observe overtones in the mid IR. Overtones are easily identified in the near IR, which is the region of the electromagnetic spectrum above 4000 cm^{-1}.

Different vibrational modes can also couple to produce combination bands. These bands may appear in different regions of the spectrum but are easily identified in the near IR region. Recent technological developments in the construction of detectors and light sources have motivated the use of near IR spectroscopy to interrogate the signature of chemicals by examining overtones and combination bands. In this section we report on the first measurements of the near IR of TNT.

The near IR spectra of the two forms of TNT are displayed on figure 3.1. The corresponding Normaski images are indicated at the right hand side of each spectrum. The spectrum of the non crystalline form of TNT between 4000 and 6500 cm-1 is structure less. The spectrum of the needle like form of TNT has a rich near IR structure in the combination and first overtone regions. Assignment of the transitions that give rise to the different bands in the near IR spectrum of the needle form of TNT is beyond the scope of this work. However it is of interest to discuss the possible reasons for the marked difference between the spectra of the non crystalline and needle like forms of TNT. The lack of bands in the near IR of the non crystalline form of TNT likely results from poor coupling of vibrational states due to the higher degrees of freedom in the molecules- as opposed to the crystalline state, where molecular motion is limited.

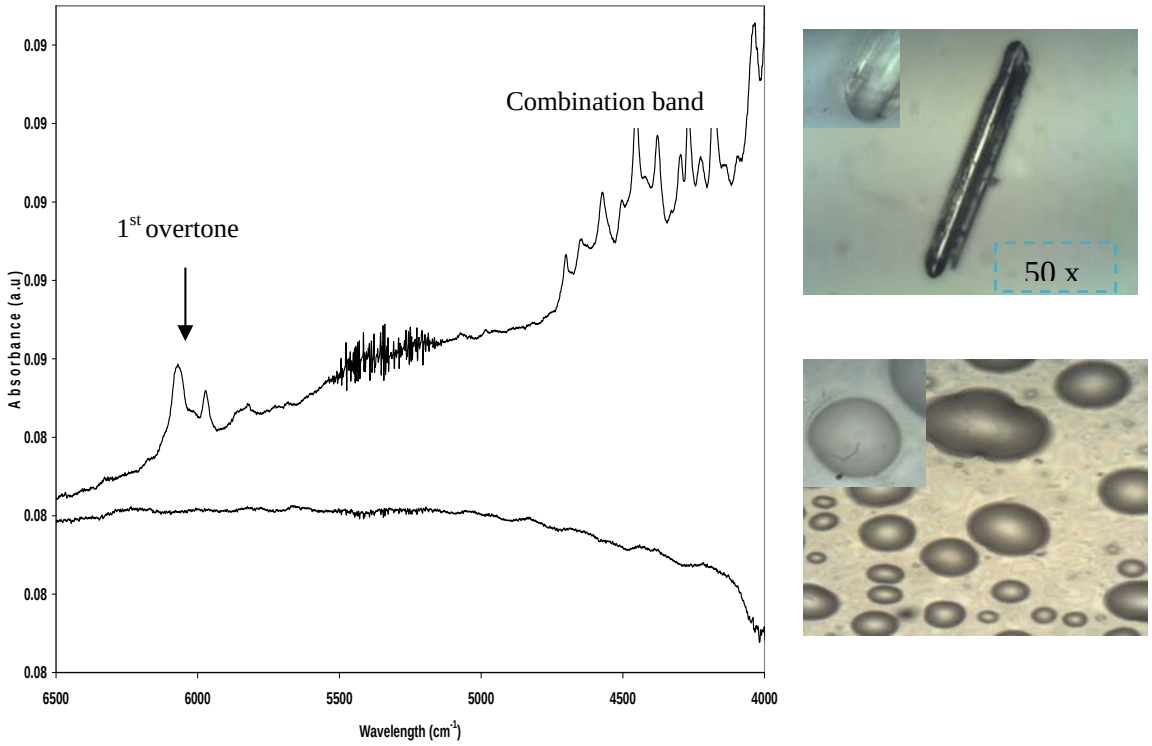


Figure 3.1: TNT Crystalline and non Crystalline NIR spectra.

3.2 DLC and NIR of TNT

We have extended the use of diffracted light microscopy to the characterization of crystalline and non crystalline TNT. The images reveal the formation of well defined TNT needles upon drying deposits from concentrated solutions. The images of the non crystalline TNT reveal the formation of 3 dimensional spheres of TNT. Near IR measurements are also used to differentiate among the two forms of TNT.

3.2.1 Traces of TNT

A microscope slide was placed in the sample compartment of the Nikon microscope equipped with a curved interference for DLC imaging. The slide was focused with a 50 x Olympus LM Plan F1 objective. TNT in toluene (0.001 M) was deposited on the slide in amounts of 6 μL until a final volume of 0.5 mL was reached. Figure 3.2 summarizes a sequence of images taken while the deposit is dryed on the microscope slide surface. The image clearly shows the formation of well defined "drops" of TNT between t=2 and 24 hours after the sample was prepared. In contrast to previous works, the islands appear to have 3 dimensions when examined with DLC as oppose to the flat spots that have been reported in previous works.

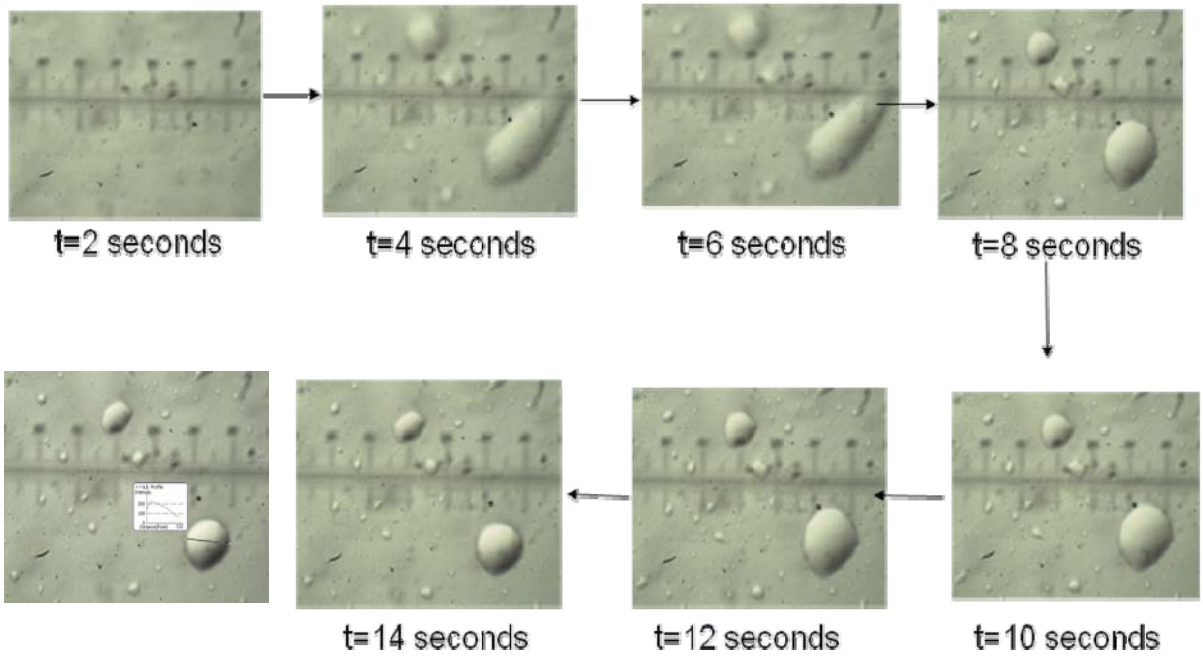


Figure 3.2: TNT sequence of crystal formation dependent of amount of TNT.

The non-crystalline form exhibits a morphology that resembles a drop. The circles and squares on figure 3.3a represent the average number of small particles and large islands as a function of deposit drying time. We have cut the large islands as those formations that are larger than 3 μm while the small particles are formations that can be identified in the images that are smaller than 3μm. The large islands appear first in the drying process while the smaller particles appear at slightly longer times. The average number of large islands does not change much over time while the numbers of small particles appear in the first 15 seconds of preparation of the deposit: it increases by less than 1% for drying times that correspond to a 24 hour period.

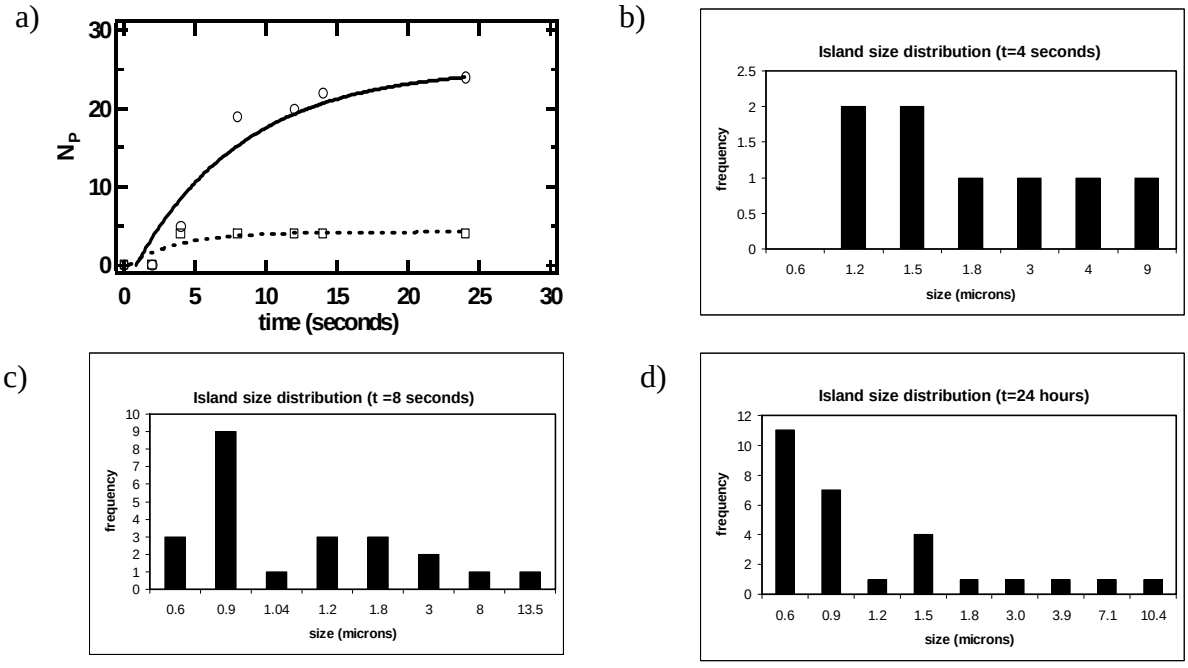


Figure 3.3: (a) The circles and squares represent the average number of small particles and large islands as a function of TNT/toluene drying time. The particle size distribution is illustrated for (b) 4, (c) 8 and (d) over 50000 seconds on the same figure.

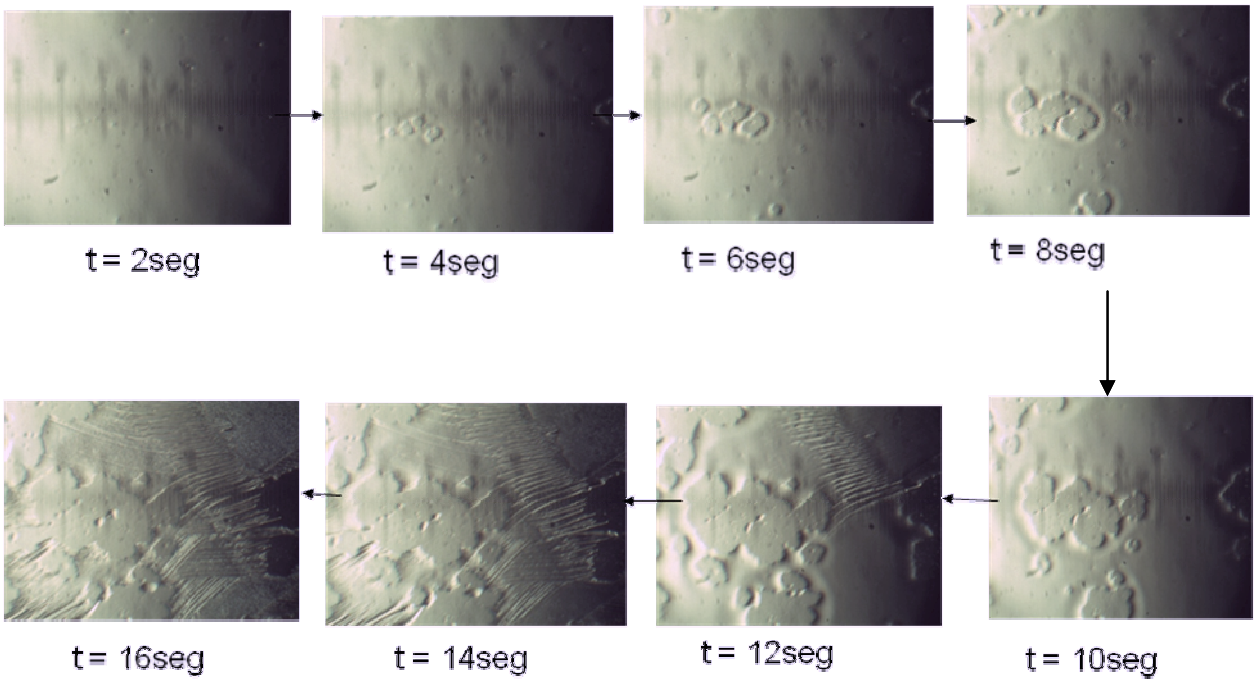
The particle size distribution was also found to depend on drying time. The particle size distribution that corresponds to drying times of (b) 4, (c) 8 and (d) over 50000 seconds are indicated on figure 3.3. The particle size distribution of the islands with diameters larger than

3mm does not change significantly with time. A marked change with time is observed in the distribution of the smaller particles (d<3 mm). This change is attributed to the evaporation of traces of solvent with time that leaves small amounts of TNT deposits scattered over different regions of the surface.

3.2.2 Larger amounts of TNT

The effect of mass in the formation of solid forms of TNT was also investigated. TNT in toluene (0.001 M) was deposited on the slide in amounts of 6 μL until a final volume of 0.7 mL was reached. Figure 3.3 summarizes a sequence of images taken while the deposit is dried on the microscope slide surface. The image clearly shows the formation of well defined "crystals" of TNT between t =2 seconds and 24 hours after the sample was prepared. The TNT mass on the slide surface is about of 1.6×10^{-4}g of the total solution.

There is no significant observation in the first 2 seconds after the formation of the deposit. A small structure can be identified in the middle of the image about 4 seconds after the deposit is prepared. The size of the structure continues to increase with drying time: similar structures can be identified in images of deposits that are dried for about 8 and 10 seconds. Well defined one-dimensional structures that resemble needs are observed when drying times of 12 to 16 seconds are reached.



To gain insight into the chemicals that give rise to the different structures imaged in the DLC measurements we measured the intensity of the transmitted light in selected regions of a deposit allowed to dry for 16 seconds. The left and right hand side of figure 3.4 summarizes images obtained at t=16 seconds in the DLC and non DLC modes, respectively. The insert represent the intensity of the light transmitted along the indicated vertical lines. In the images obtained in the DLC mode we observe that the transmitted light intensity fluctuates a little: the sharp and well defined peak observed occurs at the interface between the center structure and a group of needles (see the arrow). The structure less intensity profile suggests that the diffracted light used for the DLC measurements is not sensitive enough to identify chemical species. Rather, the significant changes in the intensity profile appear to result from changes in the index of diffraction across the sample. This change in index of diffraction likely results in the 3 dimensional appearances of the images.

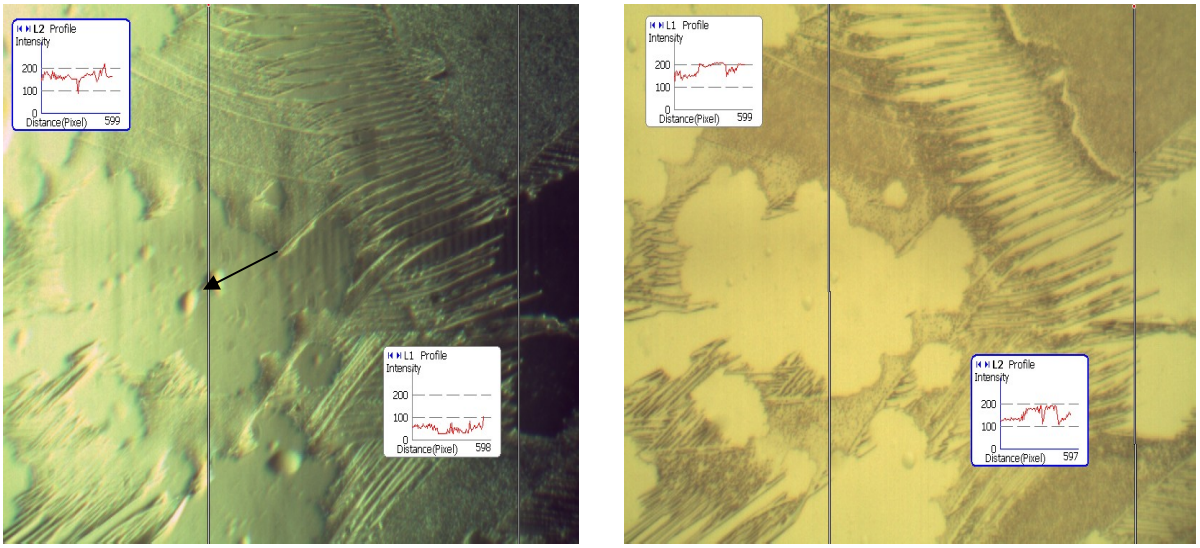


Figure 3.5: DLC images with graphic relation of light intensity vs. distance.

Turning to the intensity profile of images obtained in the non DLC mode, it is possible to observe well defined variations in the intensity of the light transmitted. We turn our attention to the intensity profile along the line labeled ‘a’. As we move from the top towards the bottom of the image it is possible to identify a region where the light intensity remains nearly constant and then an increase in the intensity of light as we move toward the bright region. The intensity of the light decreases again when we reach the dark region and increases as we approach the end of the line at toward the bottom of the image.

The needle form of TNT is the most stable form of the explosive. The left and right hand side of figure 3.6 illustrates DLC and non DLC images of a selected region of the sample rich in TNT needles. As we move along the horizontal or vertical lines, the intensity of the light transmitted increases in those regions where the needles are found. In contrast, the intensity of the light transmitted decreases when the TNT needles are on the way of the light path in non DLC imaging measurements. These results are consistent with our claim that the 3

dimensional appearances attained in DLC imaging measurements results from changes in the diffraction index of the material as oppose to traditional measurements, where the object imaged serves as interference due to light absorption.

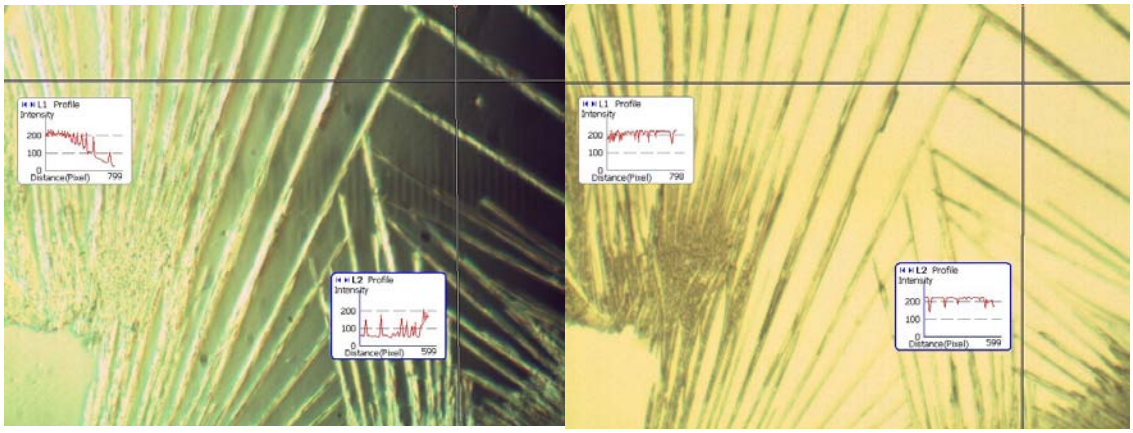


Figure 3.6: The left and right hand side illustrates DLC and non DLC images of a selected region of the sample rich in TNT needles. The inserts represent the light intensity profile obtained along the vertical or horizontal lines in the images.

3.3 Exposure to environmental conditions

It is of interest to examine the TNT that has been exposed to typical environmental conditions. Under typical environmental conditions, TNT used in terrorist attacks, will likely be expose to sunlight and water. These factors will likely cause chemical changes and variation in the morphology and spectroscopy signature of the explosive.

The TNT powder employed for the measurements is a white solid as illustrated on the image displayed on the left hand side of figure 3.7. It turns colored-yellowish- upon exposure to sunlight for about 3 to 5 minutes. In solution, on the other hand, the changes in color depend on the nature of the solvent. Solutions of TNT in aprotic solvents-like toluene- remain clear after sunlight exposure for several minutes. Solutions of TNT in protic solvents, on the other hand, change turn colored-reddish- upon sunlight exposure within a minute.

We have extended near IR and DLC imaging measurements to deposits of TNT exposed to sunlight with the purpose of contributing to the body of knowledge related to the physical properties of TNT exposed to environmental factors.

Ultraviolet-visible absorption spectroscopy measurements allow to quantitatively establish the effect of sunlight exposure on TNT. Figure 3.9 shows the UV-visible absorption spectra of TNT in water before and after exposure to sunlight for 3 minutes. The onset of light absorption of the solution of TNT in water is around 440 nm. Light absorption increases with decreasing wavelenth towards the UV region. The spectrum of TNT in water solutions exposed to sunlight is markedly different. It is characterizaed by a well defined and strong band in the visible centered around 520 nm and a region of strong light absorption in the UV region.

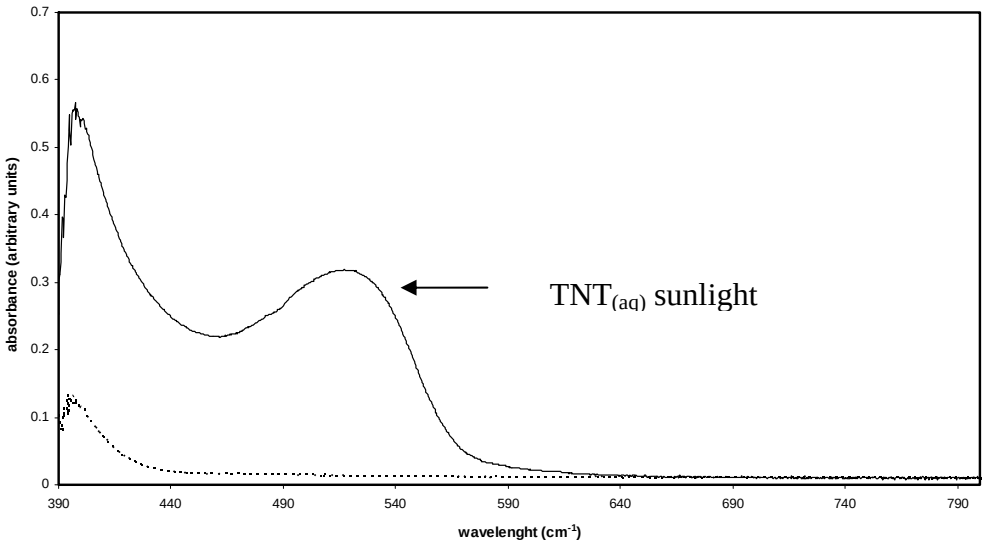
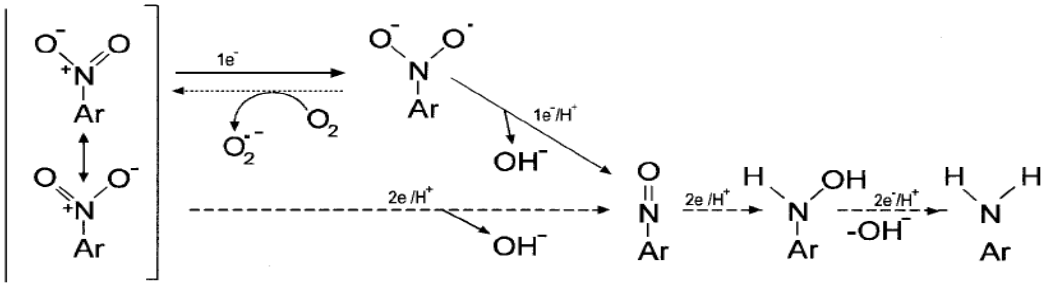


Figure 3.9: UV-VIS spectrum of sunlight exposed TNT dissolved in water and toluene

Photoreduction of nitroamines in protic solvents like water is well documented. As illustrated in scheme 3.1, it proceeds through the reduction of the nitrogroup from the stepwise protonation and subsequent loss of OH- groups. Amines are the final product of the reduction of nitroaromatics. Amines are colored and absorb light between 500 and 600 nm, in the region of the electromagnetic spectrum where the sunlight exposed TNT in water solutions are established to absorb. In passing, we note that aprotic solvents cannot reduce

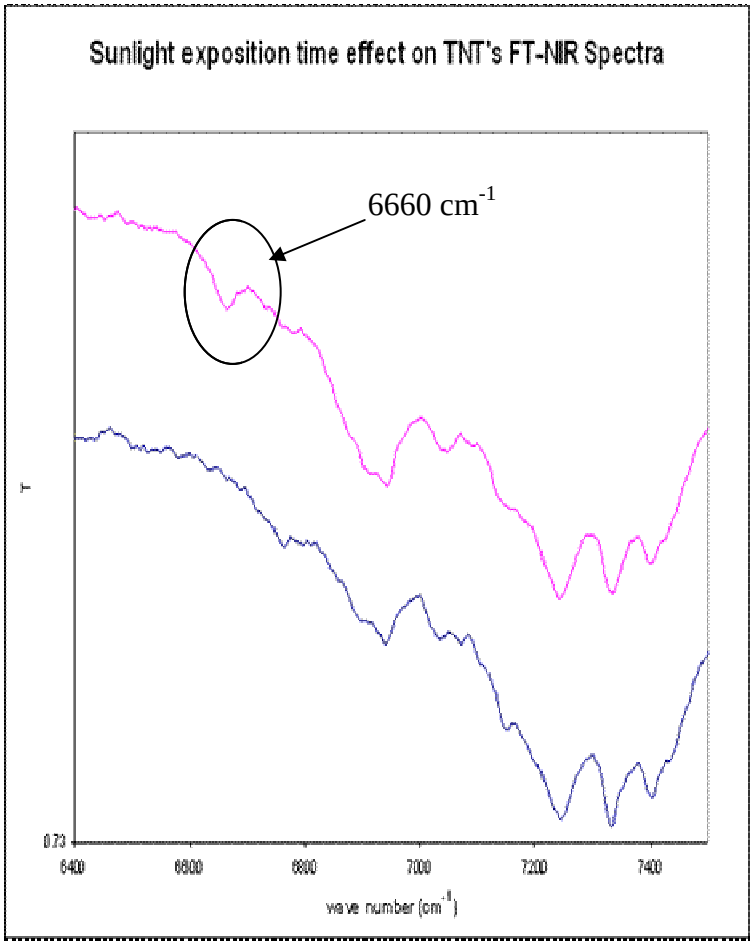
the nitro group. TNT in toluene, for instance, cannot be photoreduced under our experimental conditions.



Scheme 3.1: Mechanism of photodecomposition reaction for the conversion of nitro groups to amines.

3.5 NIR Spectra of sunlight exposed TNT

Real time detection of explosives in mines and improvised explosive devices relies on obtaining a useful fingerprint for TNT exposed to the different environment conditions. In general, the spectroscopic signature of powder forms of TNT are more important than the corresponding signature of other forms of the explosive. Figure 3.10 compares the spectroscopic signature of powder TNT before and after exposure to sunlight between 5400 and 7400 cm^{-1}. This region is traditionally regarded as the one that corresponds to the first overtone region. The spectrum of the powder before exposure to sunlight is characterized by bands between 7000 and 7500 cm^{-1}. A new band centered at 6660 cm^{-1} is readily identified in the spectrum of sunlight exposed TNT. This band was not found to change significantly in intensity or shift in frequency with increasing sunlight exposure time. The first overtone region occurs when Δn is 2. Therefore, the allowed transition is expected to occur at (6660 cm^{-1})/2 which corresponds to a frequency of 3330 cm^{-1}. This region of the spectra is usually associated with vibrational modes of amines. Indeed, the NIR of amines, in particular secondary amines, is consistent with a well defined band around 6400 to 6800 cm^{-1}.

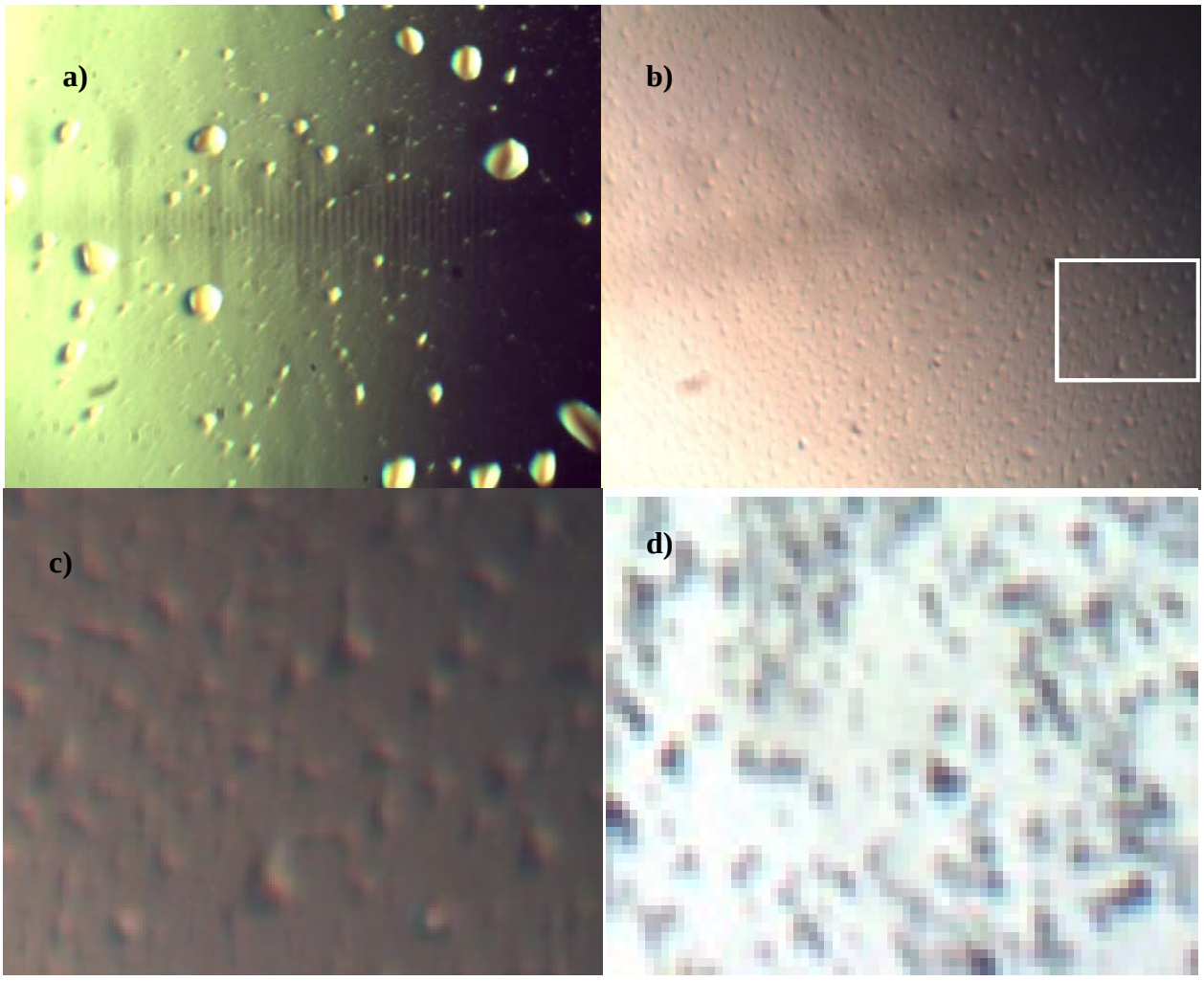

Sunlight exposition time effect on TNT's FT-NIR Spectra
6660 cm-1
T
0.73
6400
6600
6800
7000
7200
7400
wave number (cm-1)

e. A
ısistent

3.6 DLC Images of deposits of sunlight exposed TNT

DLC images of deposits prepared from solutions of TNT in toluene and TNT in water exposed to sunlight are displayed on figure 3.11. Images of solutions of TNT in toluene always have a green color, like the image displayed on figure 3.11a. This color does not represent the formation of a new photodecomposition product: the reader should recall that the UV-visible absorption spectra of solutions of TNT in toluene remains do not change significantly upon exposure to sunlight. The green color results from emission of the TNT due to the use of the well focused light of the microscope.

Aqueous solutions of TNT exposed to sunlight become red upon exposure to sunlight. Deposits of aqueous solutions of TNT exposed to sunlight are displayed on figure 3.11b. The red color of the deposit is easily spotted at the left hand side of the image, a little bit off the strong DLC region that is observed at the right hand side. The particles still look red in the DLC region, as evidenced in the image displayed on figure 3.11c, which represent the region indicated by the square in 3.11b magnified 800 times. The red color can be easily spotted in the individual particles and the 3 dimensional structure of the deposit is easily observed. In passing, crystals in powder samples of TNT exposed to sunlight were also imaged under DLC conditions. The red color observed in the smaller particles is also observed in the crystal 3.11d, although the color is not uniform across the crystal.


a)
b)
c)
d)

CHAPTER 4

Conclusion

After remarks in all the experimental procedure, we seek out our goals to conclude this recent effort. First, we prove that interference can be used to generated collimated light, which let us confirm a new technique for commonly used microscope knows as Diffracted Light Contrast. This method generated high quality images, increasing the resolution and tridimentionallity of our sample, and creating a cost effective method with an easy to acquire.

We conclude in our case of study with 2,4,6 trinitrotoluene (TNT), based on the UV-VIS spectrum and Near IR spectrum. The UV-VIS spectrum exhibit bands in the visible region with TNT exposed with sunlight, which are not observed in TNT not exposed to sunlight. We can conclude that a chemical changes has occurs in TNT structure. Near IR spectrum reveals that it is sensitive to the TNT structure. We obtained an important effect of sunlight exposure with TNT, which has the chemical change in structure when is exposed with sunlight radiation. We confirm this chemical changes with near IR spectrum with spectrum signal of formation of amines by photodecomposition. The near IR is consistent with the formation of amines in the photolysis of TNT in protic solvents.

CHAPTER 5

Future Works

The develop of this effort can be an useful tool in others science fields. For example, biology, engineering, agronomy and other branch that requires the microscope as a tool.

DLC could be a great tool in biology field. Most of the experimental procedure in biology requires microscopy as a main tool for characterization and morphology identification. Sometimes the experimental procedure requires of advanced techniques to obtain detail about some structure. But, those advanced technique that will destroyed the sample. DLC provides the advantage of study the sample without uses of organic tints, or microscopy technique that uses some sources that could be damage to the sample.

When we are study microscopic living things, most of these species tends to be transparent or with no color. Those species can be identified with the used of light contrast. One of the advantages of DLC is the possibility of identified organism that has no color with the contrast that is produced when the diffraction pattern is produce into the sample. When we are working with living things, we have to be present that living things are not easy to obtain as chemical compounds. The experimental process with living things could take weeks to developed and most has a lifetime period, that limit works time.

CHAPTER 6

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