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Development of an optical measurement method for "sampled" micro-volumes and nano-flow rates

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Abstract

Radiopharmaceuticals used in nuclear medicine for therapy or diagnosis (molecular imaging, PETscan, scintigraphy) are characterized in terms of volume activity before injection to patients. The current measurement process relies on dose calibrators which have to be calibrated by transfer standards, traceable to primary standards. For very short half-life radionuclides (few minutes), the metrological traceability can only be assured through an on-site calibration with primary standards. However, until now, there is no primary system for the direct measurement of such high activity radioactive solutions. This study presents the sub-system under development for the measurement of a sampled volume of the order of one microliter with an associated relative target standard uncertainty of 1 %.
This article focuses on the volume measurement method development and its validation by comparison to the gravimetric method.
The paper, in a first part, describes the developed method and the associated hardware and software. The authors have chosen a non-contact optical method implemented by a microscope camera and associated optics in front of a transparent capillary.
The second part of the paper describes the measurement process. Several image processing steps are described and the traceability to dimensional units are presented.
Finally, the paper presents some validation results by comparison to a gravimetric measurement, including repeatability and accuracy tests. Further development and improvements, necessary for the finalization of the prototype and the measurement of nano-flow rates are discussed.

1. Introduction

1.1 General context

Low liquid flow rates, typically below 1 $l.h^{-1}$, are present in a growing number of applications. For example, precise dosing in pharmaceutics, chemical or food industries, microfluidics and its applications, therapeutical treatments, fuel and addtitives injections in automotive industry. All of these applications require dedicated measurement methods and instruments, given the increased amount of complexity of Physics underlying flows at such scales. Indeed, the flows of liquids at these flow rates are subject to additional influence parameters, such as evaporation, surface tension variations, adsorption, etc.
Since the beginning of the 2010s, some national reference laboratories in flow metrology, including the CETIAT, have reference calibration benches. Most of these laboratories allow the measurement of flow rates down to a few microliters per hour.
However, below this threshold, metrological traceability to the International System of Units (SI) becomes difficult due to the lack of flow references.

1.2 Context at CETIAT

CETIAT's Liquid Flow Measurement Laboratory is the national reference laboratory, associated with the National Laboratory for Metrology and Testing (LNE) for the development of the "liquid flow" standard for water. Until 2012, the LNE-CETIAT was able to perform flow rates calibrations for flow rates ranging from 8 $l.h^{-1}$ to 36 $m^3.h^{-1}$. This laboratory provides the best uncertainties in France for this physical quantity.

In 2012, the laboratory extended its capabilities and inaugurated a new calibration and testing platform for the generation and measurement of a micro-liquid flow over a range of flow rates from 1 $ml.h^{-1}$ to 10 $l.h^{-1}$ [1,2] . This platform, dedicated to calibrations and tests of measuring devices used in the pharmaceutical, medical, fine chemicals and analysis fields, is unique in the world because of its technical and metrological capabilities. It has been accredited by COFRAC (French accreditation body) for calibrating liquid flow meters. Moreover, LNE-CETIAT micro-flow facility has been used to metrologically assess drug delivery devices such as syringe pumps, in the scope of the European Joint Research Project "Metrology for Drug Delivery" funded by the EURAMET EMPIR program and the French Metrology [3]. The following figures 1 & 2 present a diagram and a picture of the French micro flow standard at LNE-CETIAT.

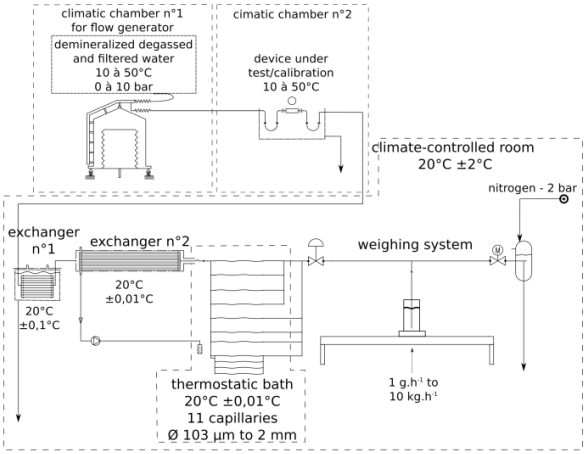


Figure 1: diagram of LNE-CETIAT micro flow facility

Figure 2: picture of LNE-CETIAT micro flow facility inside the climate-controlled room

1.3 A specific need for sampled micro-volume traceability: radiopharmaceuticals volume activity measurements

Radiopharmaceuticals are pharmaceutical drugs containing radioisotopes. They can be used as diagnostic or therapeutic agents [4-9].
The activity measurement of a radiopharmaceutical solution can be realized using several methods depending on the properties of the radionuclide in question (Its physical state, type of decay, etc.). These measurements can be primary or secondary. The latter ones are generally performed in an ionization chamber. This kind of devices requires a prior calibration consisting of the determination of the calibration factor, which ensures the conversion of the ionization courant's intensity to a measured activity. This factor depends on many parameters e.g. the used source's geometry (syringe or vial), the chamber's environment, etc. Hence, a calibration factor is valid only for defined conditions and the calibration of the device in an accredited laboratory should be performed under identical or at least closest conditions to the ones in the medical service where the radiopharmaceuticals are prepared. Primary measurements of the activity of radioactive solutions emitting beta or alpha radiation are mainly performed using liquid scintillators. For this method, a preparation of radioactive primary solutions, by mixing the dissolved radionuclides with a scintillator, is necessary. For certain short-lived radionuclides used for PET in a medical service, for example ^{11}C (20 minutes) or ^{15}O (2 minutes), it is very difficult to route the radioactive solution for a primary calibration in the laboratory. This calibration must therefore be done on site. In the case where the primary measurement system is transportable, there is also a need to implement the specific material for the preparation of primary sources (e.g. precision microbalance), which is difficult to adapt to the operating constraints of a nuclear medicine service. A major technological breakthrough would be then to perform these measurements on site, with primary methods and without the need for source preparation.
In this context, this research project aims to develop an autonomous measuring device that can be used in situ by producers and radiopharmaceuticals users (radioisotope production centers for medical use and nuclear medicine services), and allowing the direct measurement of radiopharmaceuticals activity, without manipulation of the radioactive solution. The device consists of a volume sampling system, an optical sampled volume measuring system, and an activity measurement system. The solutions

usually have a high specific activity (up to 25 MBq/g for injectable solutions and a few tens to a few hundreds GBq/cm^3 at the end of production and during the synthesis of the MRP), the final system must allow measurement without manipulation of radioactive solution, in order to limit the maximum dose induced to operators and ensuring the maintenance of the sterility of the solution [10,11].

2. Description of the system

As part of the development of its volume sampling system, LNE-CETIAT had to address three main points: (1) Reducing the sampled volume, in order to reduce the total activity and thus, facilitating the measurements, (2) Improving the volume measurement accuracy, (3) Limiting the manipulation of radioactive solutions. LNHB, on its side, developed an innovative activity measurement system dedicated to radiopharmaceuticals.

2.1 Sampling and measurement system

The following figures 3 & 4 describe the sampling and measurement system.
The system developed at LNE-CETIAT is composed of (see figure 4):
A: Mako G507B camera with Optem 70XL zoom, piloted by R&D Vision HIRIS software and dedicated image processing script
B: Zaber (x and z axis, horizontal plan) positioning stage used to center the capillary in the image
C: Hilgenberg 0620 fused silica (quartz glass) capillary, length 15 cm, inner diameter 1 mm, outer diameter 1.2 mm
D: vial of radiopharmaceutical solution
E: Sartorius weighing scale used for the validation, measurement range 20 g, resolution 1 µg
F: Dinolite USB camera focused on liquid level in the vial
G: 25x25 mm white backlight
H: Zaber (y axis) translation stage to move the capillary up and down
I: Cetoni Nemesys syringe pump
J: ILS 100 µl syringe
K: Cetoni 3-way valve
L: stainless steel pipe 2 mm pipe, insulated
M: activity measurement system developed by LNHB, not described in this paper.

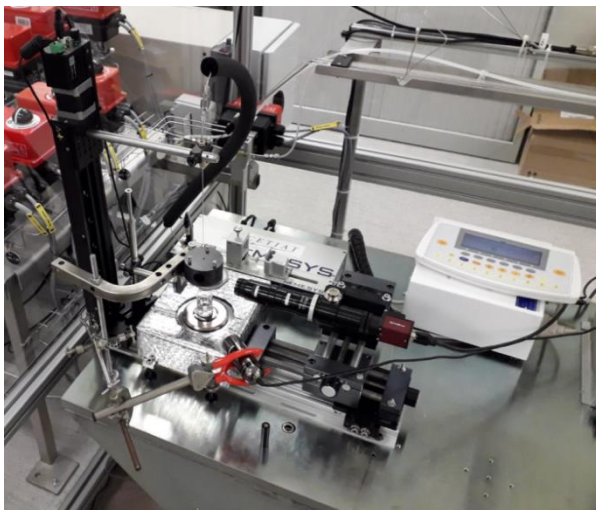


Figure 3: picture of the sampling and measurement system developed at LNE-CETIAT

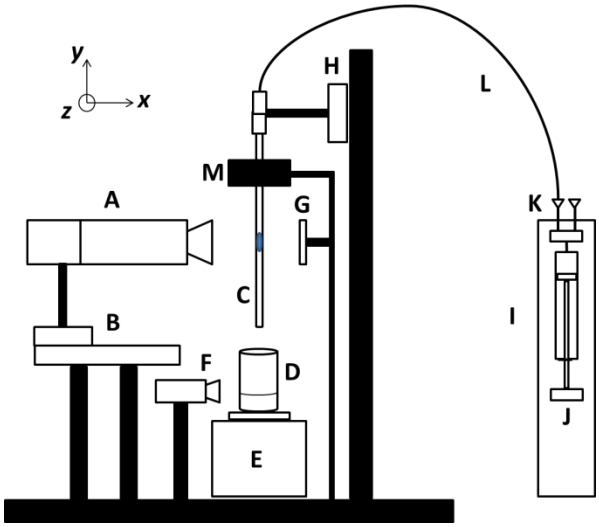


Figure 4: schematic of the sampling and measurement system developed at LNE-CETIAT

2.2 Sampling and measurement process

Using a translation stage (H), a clean and empty capillary (C) is moved toward a vial (A) containing the radiopharmaceutical solution to be calibrated (note that the weighing scale (E) is only used for validation). An USB camera (F) is used to check that the capillary end is flush with the surface: this detected by moving the capillary downward until a liquid bridge is created. Then, the capillary is immersed of a known depth: typically, for a sampled volume of 1 µl, the immersion depth is 1 mm. The capillary is then moved upward in order to break contact with the liquid and until the sampled volume appears in the image acquired by the camera (A). The syringe pump (I) is used in sucking mode to move the sampled volume a few millimeters away from the capillary end so that it is

defined by two meniscus. The figure 5 below shows the volume in position to be measured.

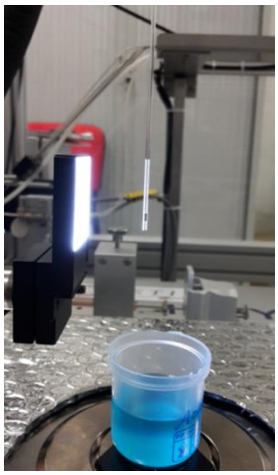


Figure 5: microvolume positioned to be measured

The figure 6 below shows the micro-volume as imaged and measured by the developed system.

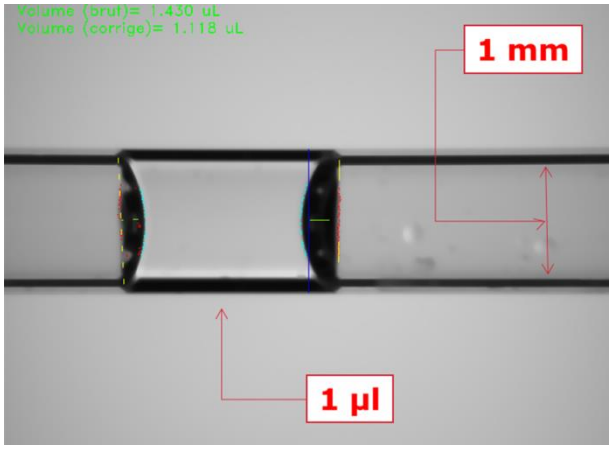


Figure 6: micro-volume as imaged and measured by the developed system

From the image acquired by the camera, several steps are required in order to measure a representative and accurate volume. Using the R&D VISION HIRIS software, a dedicated script, programed by R&D VISION to fit LNE-CETIAT's goals, performed the steps described and illustrated in the figure 7.
The image processing script only requires the inner and outer diameters as an input, and detects, measures and corrects the raw cylindrical volume formed by the meniscus edges. Several corrections, described in the following chapter, can then be applied.

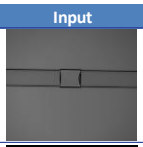
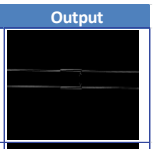
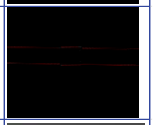
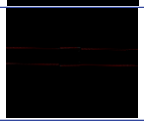
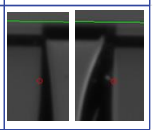
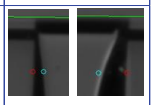
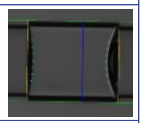
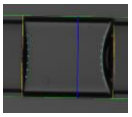
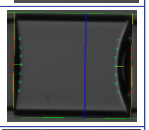
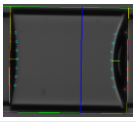
Step	Description	Input	Output
Edge detection	Oriented gradient calculation to detect horizontal borders		
Lines extraction	sharpening and detection hough line		
External edges extraction	external drop edges detection		
Spatial calibration	Minimum distance between borders calculation Zoom factor calculation relative to external diameter		
Vertical edges detection	Scan through image height to find left and right edges		
Internal edges detection	Scan through image height to find left and right edges		
External edges fitting	Linear fit on edges and minimum distance between lines calculation		
Meniscus height detection	Circular fit of internal points Finding maximum distance between edges and cirles Calculation of local radius		
Volumes calculation	Raw volume calculation : $Vb = \pi*r^2*D$ Spherical edges calculation $Vcn = 1/6*\pi*Hn*(3An^2+Hn^2)$ Corrected volume calculation $Vc = Vb - Vc1 - Vc2$		

Figure 7: image processing steps required to measure the sampled micro-volume

When a corrected volume has been measured and time-stamped by the image processing script, the capillary is translated (using stage H) upward to the activity measurement system (M) of the known distance between central focal point acquired by the camera (A) and the center of the activity measurement system.
Finally, after a few tens of seconds required to measure the activity, the capillary is translated downward, with its end inside the radiopharmaceutical vial, and the microvolume is expulsed to the vial using the syringe pump in pushing mode.
The whole sampling, volume & activity measurement duration takes less than 5 minutes.
The capillary being the only wetted part, it is replaced by new one for each measurement.

2.3 Traceability

Traceability of the measurement process is ensured by the use of a calibrated Olympus OB-M transmitted light object micrometer. Using the HIRIS software, the camera is directly calibrated so that the pixel size is known. The figures 8 & 9 illustrate the camera calibration process.

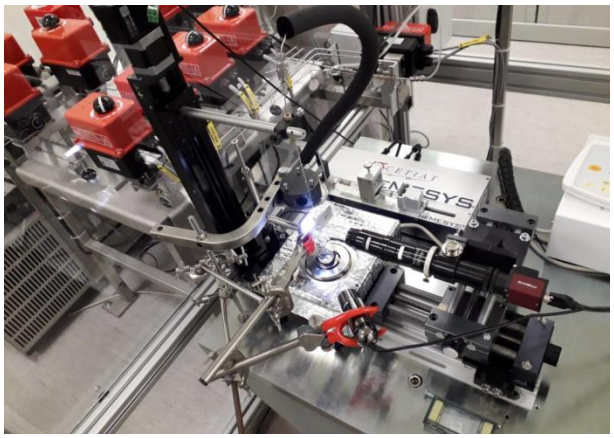


Figure 8: imaging of a micrometer object for camera calibration

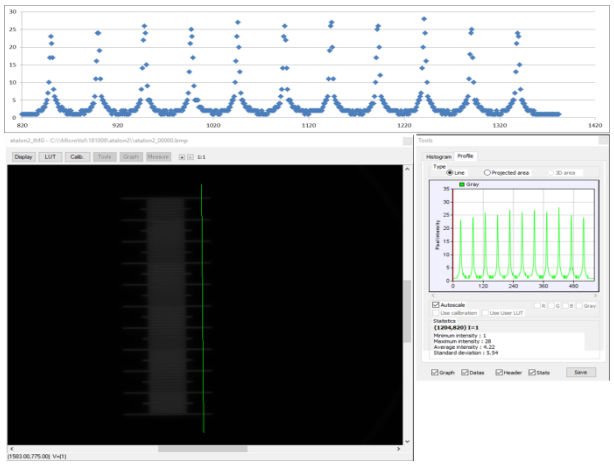


Figure 9: camera calibration using a micrometer object. Up: pixel intensity profile, Down: imaged micrometer object

When the camera is calibrated, it is then used to measure the inner and outer capillary diameter (in a location close to the image sampled volume) as the distance between edges in the intensity profiles, as illustrated in the figure 10 below.

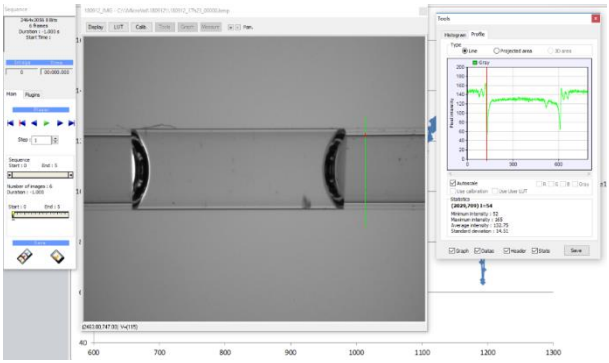


Figure 10: capillary imaging and intensity profile used for inner and outer diameter measurement

3. Results and discussion

3.1 Repeatability

In order to assess the repeatability of the measurement process, for 0.2 and 1 µl volume has been imaged and measured for different positions in the image, with voluntary eccentricity from left to right in the image. The figures 11 and 12 and table 1 below present the results obtained.

Capillary inner diameter (mm)	Position in image (pixel)	raw volume (µl)	corrected volume (µl)
1	340	1,101	0,928
1	485	1,086	0,944
1	628	1,089	0,933
1	771	1,088	0,930
1	916	1,083	0,927
1	1057	1,088	0,930
1	1210	1,089	0,930
1	1355	1,085	0,929
1	1496	1,086	0,928
1	1646	1,087	0,927
1	1792	1,085	0,934
1	1923	1,101	0,954
1	2078	1,089	0,939
1	2216	1,067	0,892

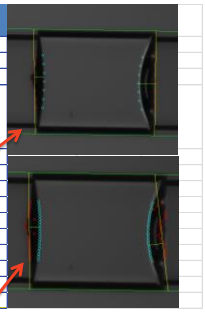


Figure 11: example of the volume eccentricity effect in the raw and corrected volume measured for a 1 µl sampled volume

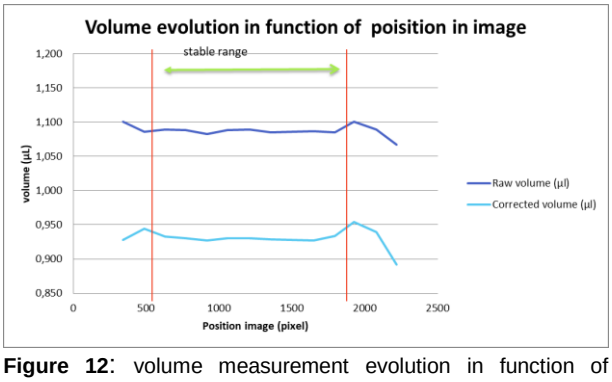


Figure 12: volume measurement evolution in function of position in image for repeatability measurements of 1 µl sampled volume

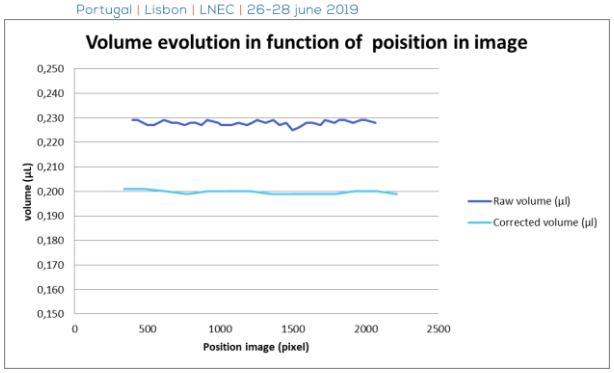


Figure 13: volume measurement evolution in function of position in image for repeatability measurements of 0.2 µl sampled volume

Capillary (mm)	Mean raw volume (µL)	Mean corrected volume (µL)	raw SD (µL)	Corrected SD (µL)	Rel. Corrected SD (µL)
1	1,087	0,930	0,002	0,002	0,26%
0,5	0,228	0,199	0,001	0,001	0,47%

Table 1: repeatability measurements results

The position repeatability results obtained show that in a reasonably wide area in the image, the relative standard deviation for 0.2 and 1 µl volume measurements are respectively 0.5 % and 0.3 %.

3.2 Accuracy

In order to assess the accuracy of the measurement process, the volume obtained by the optical method, i.e. by image processing and traceability to length unit, has been compared to gravimetric measurement using the calibrated weighing scale (E). Using a vial filled with physiological serum (liquid having physico-chemical properties close to radiopharmaceutical solutions), the weighed mass has been monitored during the sampling process described in chapter 2. The Gravimetric Regression Method (GRM) described by Liang et al. in [12], the reference sampled mass and volume has been determined with 0.1 % accuracy for a 1 µl sampled volume.
The figures below illustrate the evolution of the weighed mass during the sampling process.

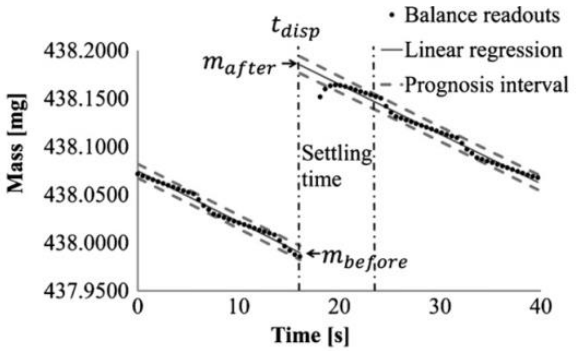


Figure 14: Gravimetric Regression Method as illustrated in [12]

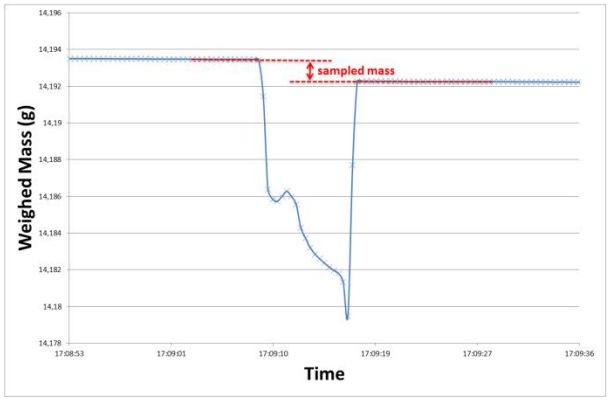


Figure 15: weighed mass during the sampling process of 1 µl

The GRM method applied here consists of (1) modelling of liquid evaporation before and after sampling, (2) calculation of average deviation between modelled mass curves, (3) conversion of sampled mass to volume using physiological serum density measured by a calibrated Anton Paar DMA5000 densitometer.
The deviation between the measured volume by the optical method and the gravimetric method is comprised between 0.2 % and 0.4 % for three measurements under productivity conditions for 1 µl sampled volumes.

4. Future developments

4.1 Capillary inner diameter measurement

As seen above, the measurement of a sampled micro-volume depends on the given outer and inner diameters of the capillary. The outer diameter can be determined with a 0.1 % relative accuracy from the intensity profile and the calibration of the camera. The inner diameter's measurement using the same method is, however, more subtle. In fact, refraction produces a pattern of shadows and bright regions on the transparent tube, preventing us from discerning the exact position of the inner diameter [13]. Other non-destructive measuring methods have then to be explored. We can mention among others, high spatial resolution X-Ray imaging techniques, like the Micro-Computed Tomography which is largely used in biology for 3D visualization of morphological structures [14] or the method using X-ray synchrotron radiation [15]. Another possibility would be to use an optical method in which the capillary is photographed using a digital camera and the focus is adjusted in such a way to produce, under certain conditions, two prominent light cusps. These cusps appear on the capillary as bright lines. The internal one corresponds to an apparent inner diameter from which the real diameter can be deduced using a mathematical equation [13]. Another way of

overcoming completely this problem would be to use a microfluidics chip in which droplets of the radioactive solution are generated using a given geometry [16] (Flow focusing, T-Junction, Co-flow focusing, etc.). In the case where the droplets do not occupy the whole section of the microfluidics channel, the value of the inner diameter is no longer necessary and the volume of the droplets can be measured simply by the optical method and a given software: Automated Droplet Measurement (ADM) [17], Droplet Morphometry and Velocimetry (DMV) [18], etc.

4.2 Optical distortion corrections

Objects inside cylindrical capillaries appear distorted when viewed from the outside, due to the refraction of the light passing through media of different refractive indices. Figure 6 shows meniscus unrealistically extended into the tube's external wall. Another alteration that may appear on a digital image is the one caused by the radial lens distortion of a camera device. Such optical distortions may cause significant errors in geometrical measurements using optical observations. The measurement of the sampled micro-volume by the R&D VISION HIRIS software can be made more precise by introducing corrections in the computer program. A method proposed by Lowe and Kutt for correcting the meniscus profile by a point-by-point spatial position correction can be used [19,20]. Also, many ways for
overcoming the lens distortion limitation exist, including camera calibration methods and distortion corrections using analytical models and computer programs [21-26].

4.3 nano-volumes measurements

The validation of the volume measurement system has been performed down to a 200 nl sampled volume (in a 500 µm inner diameter transparent capillary) with an associated relative standard uncertainty of 1%. The system described in this article is currently being upgraded with a motorized zoom so that volumes down to one nano-liter will be measured with a similar target uncertainty. The optics including the motorized zoom will be able to image a field of 2.3x1.7 mm (450 µm depth of field) down to 0.4x0.3 mm (42 µm depth of field). The figure below shows an example of a nanoliter sampled volume imaged with the current system in a 100 µm inner diameter capillary.

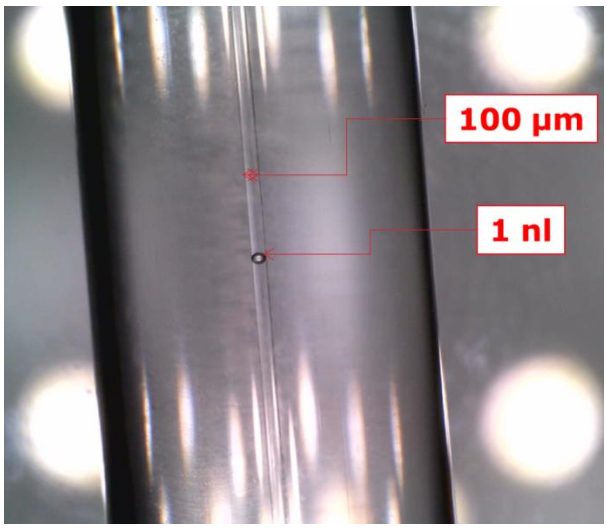


Figure 16: 1 nl sampled volume in a 100 µm inner diameter capillary imaged with LNE-CETIAT current system

4.4 flow measurements

Feasibility of low flow measurements using an optical system to track the displacement of a meniscus in a transparent capillary has already been demonstrated [27]. Using the same method, LNE-CETIAT aims at measuring flow rates down to 1 nl/h in small transparent capillaries of 100 µm inner diameter or less. A few changes in the image processing process, will allow to measure the position (in µm) of the imaged meniscus in two successive time-stamped pictures, so that the average measured volumetric flow rate Q can be expressed as

$$Q = \frac{\Delta x}{\Delta t}\pi R^2$$

With Δx the displacement of the meniscus, Δt the difference in seconds between two successive time-stamped pictures, and R the capillary inner diameter radius.

5. Conclusion

In order to improve accuracy and traceability of radiopharmaceuticals volume activity measurements, LNE-CETIAT has developed a non contact micro-volume sampling and measurement system. Using on-the-shelf parts and a dedicated image processing algorithm, the system has been validated for sampled micro-volumes of 0.2 and 1 µl with relative standard uncertainty of 1 %. Future developments will allow this system to measure volumes down to 1 nl and flow rates down to 1 nl/h.

6. Acknowledgements

This work has been performed thanks to a French Metrology national funding piloted by LNE-DRST, and in collaboration with Laboratoire National Henri Becquerel (LNHB). Future developments will be funded in the scope of the Joint Research Project "Metrology For Drug Delivery 2", funded by the EURAMET EMPIR program of the European Union and the French Metrology funding piloted by LNE-DRST.

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