

measurements are required, for example, to detect entities which are below the reported limits of detection for conventional Raman spectroscopy, such as viral particles. In this respect, Lee et al (2015) describes the use of Surface Enhanced Raman Spectroscopy (SERS) for the detection of HIV-1. SERS differs from conventional Raman and provides enhanced signals from molecules which are adhered to roughened metal surfaces at the nanoscale, such as Ag, Cu or Au. In Lee et al, an Au nanodot fabricated indium tin oxide substrate comprising bound anti-gp120 antibody fragments was used for the specific binding of HIV-1 virus-like particles, to ensure the generation of an enhanced signal for HIV-1 virus-like particle detection. SERS however, can be associated with limitations, including the requirement to pre-prepare a substrate with an appropriate immuno-interactive molecule, which limits the generic application of the technology, and the increased cost associated with this. Further, SERS, by the nature of the requirement of binding to the entity of interest, is always invasive within a sample.

Thus, further methods are required to characterise and to assess entities which may be associated with sensitive or weak signals, such as viruses, and to assess such entities *in situ*. In addition, there is a need for methods to monitor viral component abundance in real time, in order to monitor and maximise the proportion of functional viral particles being produced.

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Summary of the Invention

Surprisingly, in direct contrast to the teaching in the art, the inventors have shown that conventional Raman spectroscopy can be used to accurately and sensitively quantify viral titre in real-time. This can be achieved by directly irradiating the viral culture medium with a light source and obtaining Raman spectroscopy data as further described herein. The methods thus obviate the need for processing of the viral culture medium. The inventors have shown that viral titre as determined by conventional Raman spectra according to the methods described herein is comparable to offline titre measurements (*e.g.* as measured by offline assays such as RT-qPCR and p24 ELISA), and that conventional Raman spectroscopy used in accordance with the methods described herein can provide an alternative rapid and reliable method to assess viral titre. As discussed previously, this

finding is particularly unexpected in view of the prior art where conventional Raman spectroscopy was associated with the production of weak signals, and where the low concentration of virus in solution would have been understood to be beneath the typical lower limits of detection using conventional Raman spectroscopy, *e.g.* when applied to bulk solution systems, especially those with large scattering variation and in the presence of unwanted fluorescent background signals. This finding is also particularly unexpected in view of the prior art which requires the processing of viral culture medium to allow the use of Raman spectroscopy, for example processing of viral culture medium to concentrate viruses prior to analysis using Raman spectroscopy, or to prevent biofilm formation.

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In addition, the inventors have shown that it is possible to use conventional Raman spectroscopy to monitor viral component abundance in real time, and in particular to quantify viral nucleic acid abundance and viral structural molecule abundance in real time. Thus, Raman spectroscopy can be used to determine the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules in a sample, and thus determine the proportion of functional viral particles produced.

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The inventors have identified a series of spectral variables which are important in enabling predictions with models processing the real-time Raman spectroscopy data to achieve the measure of real-time viral titre and/or viral nucleic acid abundance and viral structural molecule abundance. The inventors have established stratified ranges of increasing numbers of variables with different importance thresholds to provide variable ranges for the accurate prediction of viral titre and/or viral nucleic acid abundance and viral structural molecule abundance when using Raman spectroscopy. The inventors have shown that Raman spectroscopy can be used to identify the start, production phase, and end of the viral production process.

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| The present [invention-disclosure](#) thus relates to the use of Raman spectroscopy for the monitoring and/or assessment of viral nucleic acid abundance and viral structural molecule abundance in a sample. Alternatively viewed, the [invention-present disclosure](#) relates to a method for monitoring and/or assessing viral nucleic acid abundance and viral structural

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molecule abundance in a sample, comprising the steps of analysing a Raman spectrum of a sample comprising virus

using a multivariate model and determining viral nucleic acid abundance and viral structural molecule abundance. Such a method may additionally comprise a step of carrying out Raman spectroscopy on the sample, *i.e.* to obtain the spectrum.

5 The present ~~invention-disclosure~~ further specifically relates to the use of Raman spectroscopy for the monitoring and/or assessment of the start, production phase, and/or end of the viral production process. The inventors have identified that Raman spectroscopy can be used for the determination of viral nucleic acid abundance and viral structural molecule abundance, as discussed above, and particularly the inventors have
10 identified specific wavenumber ranges which require assessment for this purpose. The intensity of such peaks may be determined in a method of the ~~invention~~present disclosure, where the intensity of such peaks may result in the production of a fingerprint which can be assessed with a multivariate model to determine viral nucleic acid abundance and viral structural molecule abundance. In a preferred embodiment of the ~~invention-present~~
15 disclosure the viral nucleic acid abundance and viral structural molecule abundance which is monitored and/or assessed is adeno associated virus viral nucleic acid abundance and adeno associated virus viral structural molecule abundance .

One advantage of the present ~~invention-disclosure~~ is that viral nucleic acid abundance and
20 viral structural molecule abundance, and the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules, can be continuously monitored in real-time. There is no need to process samples from the viral culture medium to generate an estimate of viral nucleic acid abundance, viral structural molecule abundance and the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules.
25 Measurements may be made *in situ*, if desirable. In other words, measurements may be made directly on the viral culture medium in the growth incubator. Measurements may be made *ex situ*, if desirable. In other words, measurements may be made directly on the viral culture medium in an aliquot of the viral culture medium taken from the growth incubator or separated from the main chamber of the growth incubator. Whether measurements are
30 made *in situ* or *ex situ*, measurements may be made directly on the viral culture medium without the need for further processing of the viral culture medium. This type of approach is sometimes described as being ‘in-line’ or ‘at-line’ analysis. Thus, more accurate trends

in viral nucleic acid abundance and viral structural molecule abundance can be produced, without risk of contamination. The methods of the present ~~invention~~ disclosure are thus faster and simpler than conventional off-line methods which require processing of viral culture medium. Particularly, the production stage of the culture can be much more accurately measured, leading to a more accurate timing of the end/harvesting stage of viral production, allowing process cessation at an appropriate time point, potentially reducing the cost of the production process.

Therefore, in a first aspect, the ~~invention~~ present disclosure provides: a method of determining in a sample using Raman spectroscopy the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules, the method comprising the steps of:

- (a) providing a sample and irradiating the sample with a light source;
- (b) (i) measuring the total intensity of Raman scattered light within each one of a first plurality of wavenumber ranges to obtain a first wavenumber intensity data set for the sample, wherein the first plurality of wavenumber ranges are pre-selected and are characteristic of viral nucleic acids in the sample;
- (ii) performing a first set of mathematical data processing steps on the first wavenumber intensity data set; and
- (iii) determining the viral nucleic acid content of the sample based upon the output of the first set of mathematical data processing steps;
- (c) (i) measuring the total intensity of Raman scattered light within each one of a second plurality of wavenumber ranges to obtain a second wavenumber intensity data set for the sample, wherein the second plurality of wavenumber ranges are pre-selected and are characteristic of the one or more viral structural molecules of the viruses in the sample;
- (ii) performing a second set of mathematical data processing steps on the second wavenumber intensity data set; and

- (iii) determining the content of viruses comprising the one or more viral structural molecules in the sample based upon the output of the second set of mathematical data processing steps; and

- defined methods, preferably wherein each step is performed by the same method. In any such method the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules may be determined repeatedly over a time period to provide a measure of the change in the ratio in real time. The change in the ratio in the sample may be used to
- 5 determine the start phase, the production phase and/or the stationary phase of a viral production process. Any such method may be used to determine the optimal conditions for a viral production process. Any such method may be used to assess a process downstream of a viral production process.
- 10 Any of the above-defined methods may comprise a step of comparing the ratio thereby obtained with the ratio obtained from the same sample by an alternative method, optionally wherein the alternative method is qPCR, RT-qPCR, ELISA or by visual determination by transmission electron microscopy.
- 15 [In a second aspect, ~~The invention present disclosure also~~](#) provides a method of determining the extent of viral infection in an individual using Raman spectroscopy, the method comprising determining the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules in a sample by performing the method of any one of the
- 20 [above-defined methods](#)[first aspect](#), wherein the sample is a sample which has previously been obtained from the individual. The sample may be a sample of blood, saliva, sputum, plasma, serum, cerebrospinal fluid, urine or faeces. In any such method the ratio in the sample from the subject may be compared with one or more ratio measurements which have previously been obtained for the infection in the individual, in order to provide a prognosis of the stage of infection in the individual.
- 25 [In a third aspect, ~~The invention present disclosure additionally~~](#) provides a method of determining in a sample using Raman spectroscopy the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules, the method comprising the steps of:
- 30 (a) (i) providing a first wavenumber intensity data set for the sample, wherein the first data set has been obtained by irradiating the sample with a light source and measuring the total intensity of Raman

scattered light within each one of a first plurality of wavenumber ranges, wherein the first plurality of wavenumber ranges in the

In a fourth aspect, the invention present disclosure further provides the use of Raman spectroscopy for determining the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules in a sample. In any such use, the ratio may be determined based upon measurements of the intensity of Raman scattered light obtained from the sample following irradiation of the sample with a light source, wherein the intensity of Raman scattered light is measured from a first plurality of wavenumber ranges in a Raman spectrum which are characteristic of viral nucleic acids in the sample and from a second plurality of wavenumber ranges in a Raman spectrum which are characteristic of the one or more viral structural molecules of the viruses in the sample.

In any such use the sample may be a viral culture, optionally wherein the viral culture is comprised in a bioreactor. The step of measuring the total intensity of Raman scattered light may be performed directly on the medium of the viral culture (*in situ*). Alternatively the step of measuring the total intensity of Raman scattered light may be performed directly on an aliquot of the medium which has been taken from the viral culture (*ex situ*).

In any such use the ratio in the sample may be determined at a first time point and at one or more later time points, and wherein the change in the ratio in the sample between time points is calculated. In any such use the ratio in the sample is quantified repeatedly to provide a measure of the change in the ratio in real time. In any such use the viral titre in the sample may be quantified by performing any of the methods described and defined herein.

In any of the above-defined methods or uses, the viruses in the sample may not be HIV-1 or HIV-1 virus-like particles (HIV-1 VLPs).

In any of the above-defined methods or uses the Raman spectroscopy may not be surface enhanced Raman spectroscopy.

In a fifth aspect, the invention present disclosure further provides a method of building a multivariate data processing model which is capable of determining the content of viruses comprising one or more viral

wherein the content of viruses comprising one or more viral structural molecules in a sample is determined using the model parameters obtained for the identified Raman spectral variables derived from the multivariate data processing model.

- 5 In a sixth aspect, the invention present disclosure yet further provides a method of building one or more multivariate data processing models which are capable of determining the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules in a sample from a Raman spectroscopy wavenumber intensity data set obtained for the sample, the method comprising:
- 10 (a) providing the sample and irradiating the sample with a light source;
- (b) (i) measuring the total intensity of the Raman scattered light within each one of a first plurality of wavenumber ranges to obtain a first wavenumber intensity data set for the sample wherein the first plurality of wavenumber ranges are pre-selected and are characteristic of viral nucleic acids in the sample;
- 15 (ii) measuring the total intensity of the Raman scattered light within each one of a second plurality of wavenumber ranges to obtain a second wavenumber intensity data set for the sample wherein the first plurality of wavenumber ranges are pre-selected and are characteristic of the one or more viral structural molecules of the viruses in the sample
- 20 (c) obtaining normalised wavenumber signal intensity data for the first and second wavenumber intensity data sets by pre-processing the signal intensity data using a pre-processing analytical method, such as a first derivative method, a second derivative method, a standard normal variate (SNV) method, a polynomial fitting method, a multi-polynomial fitting method, a mollifier method, a piecewise polynomial fitting (PPF) method or an adaptive iteratively reweighted Penalized Least Squares (airPLS) method;
- 25 (d) obtaining model parameters to be applied to the first and second wavenumber intensity data sets by applying to each one of the pre-processed signal intensity data sets a multivariate regression algorithm, such as a partial least squares (PLS) regression algorithm, optionally wherein the
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CLAIMS

1. A method of determining in a sample using Raman spectroscopy the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules, the method
- 5 comprising the steps of:
- (a) providing a sample and irradiating the sample with a light source;
 - (b) (i) measuring the total intensity of Raman scattered light within each
10 one of a first plurality of wavenumber ranges to obtain a first wavenumber intensity data set for the sample, wherein the first plurality of wavenumber ranges are pre-selected and are characteristic of viral nucleic acids in the sample;
 - (ii) performing a first set of mathematical data processing steps on the first wavenumber intensity data set; and
 - (iii) determining the viral nucleic acid content of the sample based upon
15 the output of the first set of mathematical data processing steps;
 - (c) (i) measuring the total intensity of Raman scattered light within each
20 one of a second plurality of wavenumber ranges to obtain a second wavenumber intensity data set for the sample, wherein the second plurality of wavenumber ranges are pre-selected and are characteristic of the one or more viral structural molecules of the viruses in the sample;
 - (ii) performing a second set of mathematical data processing steps on the second wavenumber intensity data set; and
 - (iii) determining the content of viruses comprising the one or more viral
25 structural molecules in the sample based upon the output of the second set of mathematical data processing steps; and
 - (d) determining the ratio of viral nucleic acids to viruses comprising the
30 one or more viral structural molecules in the sample based on the values determined in steps (b)(iii) and (c)(iii).

2. A method according to claim 1, wherein the steps of performing the first and second sets of mathematical data processing steps on the first and second wavenumber intensity data sets comprise:

- 5 (i) optionally normalising the wavenumber signal intensity data by pre-processing the signal intensity data using one or more pre-processing analytical methods, such as a first derivative method, a second derivative method, a standard normal variate (SNV) method, a polynomial fitting method, a multi-polynomial fitting method, a mollifier method, a piecewise
- 10 polynomial fitting (PPF) method or an adaptive iteratively reweighted Penalized Least Squares (airPLS) method;
- (ii) obtaining model parameters by applying to the wavenumber signal intensity data a multivariate regression algorithm, such as a partial least squares
- 15 (PLS) regression algorithm, optionally wherein the PLS algorithm is a nonlinear iterative partial least squares (NIPALS) regression algorithm or a neural network; and
- (iii) determining the viral nucleic acid content of the sample and determining the
- 20 content of viruses comprising the one or more viral structural molecules in the sample using the model parameters obtained by applying the multivariate regression algorithm to the signal intensity data.

3. A method according to claim 1 or claim 2, wherein the light source used to irradiate the sample is a laser and the sample is irradiated with light having a wavelength of 785nm;

4. — A method according to any one of the preceding claims, and/or wherein the Raman scattered light is detected using a charge-coupled device (CCD).

54. A method according to any one of the preceding claims 1 to 4, wherein:

(i) the first plurality of wavenumber ranges in the Raman spectrum which are measured to obtain the first wavenumber intensity data set for the sample comprises 4 or

more of the wavenumber ranges 1 to 12 as listed in Table 1 and wherein the VIP is ≥ 1.00 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 6 or more of the wavenumber ranges 1 to 12 as listed in Table 1 and wherein the VIP is ≥ 1.00 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 8 or more of the wavenumber ranges 1 to 12 as listed in Table 1 and wherein the VIP is ≥ 1.00 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 10 or more of the wavenumber ranges 1 to 12 as listed in Table 1 and wherein the VIP is ≥ 1.00 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises all 12 of the wavenumber ranges 1 to 12 as listed in Table 1 and wherein the VIP is ≥ 1.00 ; ~~or-~~

~~6. — A method according to any one of claims 1 to 4,~~

~~(ii) — wherein~~ the first plurality of wavenumber ranges in the Raman spectrum which are measured to obtain the first wavenumber intensity data set for the sample comprises 4 or more of the wavenumber ranges 13 to 22 as listed in Table 1 and wherein the VIP is ≥ 1.25 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 6 or more of the wavenumber ranges 13 to 22 as listed in Table 1 and wherein the VIP is ≥ 1.25 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 8 or more of the wavenumber ranges 13 to 22 as listed in Table 1 and wherein the VIP is ≥ 1.25 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises all 10 of the wavenumber ranges 13 to 22 as listed in Table 1 and wherein the VIP is ≥ 1.25 ; ~~or-~~

~~7. — A method according to any one of claims 1 to 4,~~

~~wherein (iii) —~~ the first plurality of wavenumber ranges in the Raman spectrum which are measured to obtain the first wavenumber intensity data set for the sample comprises 4 or more of the wavenumber ranges 23 to 30 as listed in Table 1 and wherein the VIP is ≥ 1.50 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 6 or more of the wavenumber ranges 23 to 30 as listed in Table 1 and wherein the VIP is ≥ 1.50 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises all 8 of the wavenumber ranges 23 to 30 as listed in Table 1 and wherein the VIP is ≥ 1.50 .

85. A method according to any one of [the preceding](#) claims ~~1 to 7~~, wherein:

(i) [the](#) second plurality of wavenumber ranges in the Raman spectrum which are measured to obtain the second wavenumber intensity data set for the sample comprises
5 4 or more of the wavenumber ranges 1 to 20 as listed in Table 2 and wherein the VIP is $\geq$ 1.00; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 6 or more of the wavenumber ranges 1 to 20 as listed in Table 2 and wherein the VIP is $\geq$ 1.00; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 8 or more of the wavenumber ranges 1 to 20 as
10 listed in Table 2 and wherein the VIP is $\geq$ 1.00; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 10 or more of the wavenumber ranges 1 to 20 as listed in Table 2 and wherein the VIP is $\geq$ 1.00; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 12 or more, 14 or more, 16 or more or 18 or more of the wavenumber ranges 1 to 20 as
15 listed in Table 2 and wherein the VIP is $\geq$ 1.00; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises all 20 of the wavenumber ranges 1 to 20 as listed in Table 2 and wherein the VIP is $\geq$ 1.00; [or](#)

~~9. — A method according to any one of claims 1 to 7, wherein~~

(ii) [the](#) second plurality of wavenumber ranges in the Raman spectrum which are measured to obtain the second wavenumber intensity data set for the sample comprises
20 4 or more of the wavenumber ranges 21 to 33 as listed in Table 2 and wherein the VIP is $\geq$ 1.25; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 6 or more of the wavenumber ranges 21 to 33 as listed in Table 2 and wherein the VIP is $\geq$ 1.25; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 8 or more of the wavenumber ranges 21 to 33 as
25 listed in Table 2 and wherein the VIP is $\geq$ 1.25; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 10 or more, 11 or more or 12 of the wavenumber ranges 21 to 33 as listed in Table 2 and wherein the VIP is $\geq$ 1.25; or
30 wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises all 13 of the wavenumber ranges 21 to 33 as listed in Table 2 and wherein the VIP is $\geq$ 1.25; [or](#)

~~10. — A method according to any one of claims 1 to 7, wherein~~
~~(iii) the second plurality of wavenumber ranges in the Raman spectrum which~~
are measured to obtain the second wavenumber intensity data set for the sample comprises
5 4 or more of the wavenumber ranges 34 to 40 as listed in Table 2 and wherein the VIP is $\geq$
1.50; or wherein the plurality of wavenumber ranges in the Raman spectrum which are
measured comprises 5 or 6 of the wavenumber ranges 34 to 40 as listed in Table 2 and
wherein the VIP is $\geq$ 1.50; or wherein the plurality of wavenumber ranges in the Raman
spectrum which are measured comprises all 7 of the wavenumber ranges 34 to 40 as listed
10 in Table 2 and wherein the VIP is $\geq$ 1.50.
:
~~11. — A method according to any one of the preceding claims, optionally wherein~~
the viruses in the sample are adeno-associated viruses (AAV).

15 ~~126.~~ A method according to any one of claims 1 to ~~43~~, wherein:
(i) the first plurality of wavenumber ranges in the Raman spectrum which are
measured to obtain the first wavenumber intensity data set for the sample comprises 5 or
more of wavenumber ranges 1 to 28 as listed in Table 3 and wherein the variable
importance projection (VIP) is $\geq$ 1.00; or wherein the plurality of wavenumber ranges in
20 the Raman spectrum which are measured comprises 10 or more of wavenumber ranges 1 to
28 as listed in Table 3 and wherein the VIP is $\geq$ 1.00; or wherein the plurality of
wavenumber ranges in the Raman spectrum which are measured comprises 15 or more of
wavenumber ranges 1 to 28 as listed in Table 3 and wherein the VIP is $\geq$ 1.00; or wherein
the plurality of wavenumber ranges in the Raman spectrum which are measured comprises
25 20 or more of wavenumber ranges 1 to 28 as listed in Table 3 and wherein the VIP is $\geq$
1.00; or wherein the plurality of wavenumber ranges in the Raman spectrum which are
measured comprises 25 or more of wavenumber ranges 1 to 28 as listed in Table 3 and
wherein the VIP is $\geq$ 1.00; or wherein the plurality of wavenumber ranges in the Raman
spectrum which are measured comprises all 28 of wavenumber ranges 1 to 28 as listed in
30 Table 3 and wherein the VIP is $\geq$ 1.00; ~~or~~

13. ~~A method according to any one of claims 1 to 4, wherein~~(ii) the first plurality of wavenumber ranges in the Raman spectrum which are measured to obtain the first wavenumber intensity data set for the sample comprises 5 or more of wavenumber ranges 29 to 59 as listed in Table 3 and wherein the variable importance projection (VIP) is ≥ 1.25 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 10 or more of wavenumber ranges 29 to 59 as listed in Table 3 and wherein the VIP is ≥ 1.25 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 15 or more of wavenumber ranges 29 to 59 as listed in Table 3 and wherein the VIP is ≥ 1.25 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 20 or more of wavenumber ranges 29 to 59 as listed in Table 3 and wherein the VIP is ≥ 1.25 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 25 or more of wavenumber ranges 29 to 59 as listed in Table 3 and wherein the VIP is ≥ 1.25 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 30 of wavenumber ranges 29 to 59 as listed in Table 3 and wherein the VIP is ≥ 1.25 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises all 31 of wavenumber ranges 29 to 59 as listed in Table 3 and wherein the VIP is ≥ 1.25 ; ~~or~~

14. ~~A method according to any one of claims 1 to 4, wherein~~ (iii) the first plurality of wavenumber ranges in the Raman spectrum which are measured to obtain the first wavenumber intensity data set for the sample comprises 5 or more of wavenumber ranges 60 to 81 as listed in Table 3 and wherein the variable importance projection (VIP) is ≥ 1.50 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 10 or more of wavenumber ranges 60 to 81 as listed in Table 3 and wherein the VIP is ≥ 1.50 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 15 or more of wavenumber ranges 60 to 81 as listed in Table 3 and wherein the VIP is ≥ 1.50 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are measured comprises 20 or more of wavenumber ranges 60 to 81 as listed in Table 3 and wherein the VIP is ≥ 1.50 ; or wherein the plurality of wavenumber ranges in the Raman spectrum which are

measured comprises all 22 of wavenumber ranges 60 to 81 as listed in Table 3 and wherein the VIP is ≥ 1.50 ;

5 ~~15. — A method according to any one of claims 12 to 14,~~
optionally wherein the viruses in the sample are lentiviruses.

10 ~~167.~~ A method according to any one of the preceding claims, wherein:
(i) the nucleic acid comprises a viral DNA genome or wherein the nucleic acid comprises a viral RNA genome; and/or

15 (ii) ~~17. — A method according to any one of the preceding claims, wherein~~ the one or more viral structural molecules comprises one or more viral proteins such as one or more nucleoproteins and/or one or more capsomeres, one or more viral carbohydrates, one or more glycosylated viral molecules such as a glycosylated viral protein and/or one or more viral lipids; and/or

20 (iii)
~~18. — A method according to any one of the preceding claims, wherein~~ the ratio provides a measure of functional viral titre.

25 ~~198.~~ A method according to any one of the preceding claims, wherein the sample is a viral culture, optionally wherein the viral culture is comprised in a bioreactor; Optionally wherein:

~~20. — A method according to claim 19, wherein~~

(i) the steps of irradiating the viral culture with a light source and measuring the total intensity of Raman scattered light is performed directly on the medium of the viral culture (*in situ*); or

30 (ii)

~~21. — A method according to claim 19, wherein~~ the steps of irradiating the viral culture with a light source and measuring the total intensity of Raman scattered light is performed directly on an aliquot of the medium which has been taken from the viral culture (*ex situ*).

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~~229.~~ A method according to any one of the preceding claims, the method comprising a first step of determining the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules at a first time point and one or more further steps of determining the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules at later time points, and further comprising measuring the change in the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules in the sample between time points, wherein each step is performed by a method according to any one of the preceding claims, preferably wherein each step is performed by the same method;
~~optionally wherein:~~

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~~23. — A method according to claim 22, wherein~~

~~(i)~~ the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules is determined repeatedly over a time period to provide a measure of the change in the ratio in real time;
~~and/or~~

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~~24. — A method according to claim 22 or claim 23,~~

~~wherein (ii)~~ the change in the ratio in the sample is used to determine the start phase, the production phase and/or the stationary phase of a viral production process.

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~~2510.~~ A method according to any one of the preceding claims, wherein:

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~~(i)~~ the method is used to determine the optimal conditions for a viral production process;
~~and/or-~~

26.

~~A method according to any one of the preceding claims, wherein~~

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~~(ii)~~ the method is used to assess a process downstream of a viral production process;
~~and/or-~~

~~27. — A method according to any one of the preceding claims, wherein~~

(iii) the method comprises a step of comparing the ratio thereby obtained with the ratio obtained from the same sample by an alternative method, optionally wherein the alternative method is qPCR, RT-qPCR, ELISA or by visual determination by transmission electron microscopy.

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2811. A method of determining the extent of viral infection in an individual using Raman spectroscopy, the method comprising determining the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules in a sample by performing the method of any one of claims 1 to 239(i), wherein the sample is a sample which has previously been obtained from the individual.

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2912. A method according to claim 2811, wherein:

(i) the sample is a sample of blood, saliva, sputum, plasma, serum, cerebrospinal fluid, urine or faeces; and/or

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~~30. — A method according to claim 28 or claim 29, wherein (ii)~~ the ratio in the sample from the subject is compared with one or more ratio measurements which have previously been obtained for the infection in the individual, in order to provide a prognosis of the stage of infection in the individual.

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3113. A method of determining in a sample using Raman spectroscopy the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules, the method comprising the steps of:

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(a) (i) providing a first wavenumber intensity data set for the sample, wherein the first data set has been obtained by irradiating the sample with a light source and measuring the total intensity of Raman scattered light within each one of a first plurality of wavenumber ranges, wherein the first plurality of wavenumber ranges in the Raman spectrum have been selected as characteristic of viral nucleic acids in the sample;

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(ii) performing a first set of mathematical data processing steps on the

- first wavenumber intensity data set; and
- (iii) determining the nucleic acid content of the sample based upon the output of the first set of mathematical data processing steps;
- (b) (i) providing a second wavenumber intensity data set for the sample, wherein the second data set has been obtained by irradiating the sample with a light source and measuring the total intensity of Raman scattered light within each one of a second plurality of wavenumber ranges, wherein the second plurality of wavenumber ranges in the Raman spectrum have been selected as characteristic of one or more viral structural molecules of the viruses in the sample;
- (ii) performing a second set of mathematical data processing steps on the second wavenumber intensity data set; and
- (iii) determining the content of viruses comprising the one or more viral structural molecules in the sample based upon the output of the second set of mathematical data processing steps;
- (c) determining the ratio of viral nucleic acids to viruses comprising the one or more viral structural molecules in the sample based on the values determined in steps (a)(iii) and (b)(iii).

20 [3214](#). A method according to claim [3413](#), wherein the method is performed according to the steps defined in any one of claims 2 to [3012](#).

[3315](#). The use of Raman spectroscopy for determining the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules in a sample.

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[3416](#). The use according to claim [3315](#), wherein:

(a) ___ the ratio is determined based upon measurements of the intensity of Raman scattered light obtained from the sample following irradiation of the sample with a light source, wherein the intensity of Raman scattered light is measured from a first plurality of wavenumber ranges in a Raman spectrum which are characteristic of viral nucleic acids in the sample and from a second plurality of wavenumber ranges in a Raman spectrum which

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are characteristic of the one or more viral structural molecules of the viruses in the sample,
optionally wherein: -

5 ~~35. — The use according to claim 34, wherein (i)~~ the sample is a viral culture,
optionally wherein the viral culture is comprised in a bioreactor, preferably wherein
-

10 ~~36. — The use according to claim 35, wherein~~ the step of measuring the total
intensity of Raman scattered light is performed directly on the medium of the viral
culture (*in situ*) or-

15 ~~37. — The use according to claim 35, wherein the step of measuring the total~~
~~intensity of Raman scattered light is performed directly~~ on an aliquot of the
medium which has been taken from the viral culture (*ex situ*); and/or

20 ~~38. — The use according to any one of claims 34 to 37, wherein~~
(ii) the ratio in the sample is determined at a first time point and at one or more
later time points, and wherein the change in the ratio in the sample between time
points is calculated, preferably -

25 ~~39. — The use according to claim 38, wherein the ratio in the sample is quantified~~
repeatedly to provide a measure of the change in the ratio in real time; and/or
(b)-

40. — ~~The use according to any one of claims 33 to 39, wherein~~ the ratio in the sample is
determined by performing a method according to any one of claims 1 to ~~18~~7.

30 ~~17.~~ A method or use according to any one of the preceding claims, wherein:
(i) the viruses in the sample are not HIV-1 or HIV-1 virus-like particles (HIV-1
VLPs); and/or-
(ii)

42. — ~~A method or use according to any one of the preceding claims, wherein the~~
Raman spectroscopy is not surface enhanced Raman spectroscopy.

- 5 ~~43~~18. A method of building a multivariate data processing model which is capable of
determining the content of viruses comprising one or more viral structural molecules in a
sample from a Raman spectroscopy wavenumber intensity data set obtained for the sample,
the method comprising:
- 10 (a) providing the sample and irradiating the sample with a light source;
- (b) measuring the total intensity of the Raman scattered light within each one of
a plurality of wavenumber ranges to obtain a wavenumber intensity data set
for the sample, wherein the plurality of wavenumber ranges are pre-selected
and are characteristic of the one or more viral structural molecules of the
viruses in the sample;
- 15 (c) obtaining normalised wavenumber signal intensity data by pre-processing
the signal intensity data using a pre-processing analytical method, such as a
first derivative method, a second derivative method, a standard normal
variate (SNV) method, a polynomial fitting method, a multi-polynomial
fitting method, a mollifier method, a piecewise polynomial fitting (PPF)
20 method or an adaptive iteratively reweighted Penalized Least Squares
(airPLS) method;
- (d) obtaining model parameters by applying to the pre-processed signal
intensity data a multivariate regression algorithm, such as a partial least
squares (PLS) regression algorithm, optionally wherein the PLS algorithm
25 is a nonlinear iterative partial least squares (NIPALS) regression algorithm
or a neural network, wherein a calibration is performed wherein the pre-
processed signal intensity data are compared with viral titre data obtained
for the same sample conditions using non-Raman spectroscopy methods
such as qPCR, RT-qPCR, ELISA or by visual determination by
30 transmission electron microscopy;
- (e) inferring response values using the model parameters obtained from the pre-
processed data; and

- (f) performing variable selection, optionally variable importance projection (VIP), and identifying Raman spectral variables; and
 - (g) optionally performing one or more further rounds of modelling by re-applying steps (d) to (f) and wherein unimportant variables are removed;
- 5 and

wherein the content of viruses comprising one or more viral structural molecules in a sample is determined using the model parameters obtained for the identified Raman spectral variables derived from the multivariate data processing model.

10 [4419](#). A method of building one or more multivariate data processing models which are capable of determining the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules in a sample from a Raman spectroscopy wavenumber intensity data set obtained for the sample, the method comprising:

- (a) providing the sample and irradiating the sample with a light source;
- 15 (b) (i) measuring the total intensity of the Raman scattered light within each one of a first plurality of wavenumber ranges to obtain a first wavenumber intensity data set for the sample wherein the first plurality of wavenumber ranges are pre-selected and are characteristic of viral nucleic acids in the sample;
- 20 (ii) measuring the total intensity of the Raman scattered light within each one of a second plurality of wavenumber ranges to obtain a second wavenumber intensity data set for the sample wherein the first plurality of wavenumber ranges are pre-selected and are characteristic of the one or more viral structural molecules of the viruses in the sample
- 25 (c) obtaining normalised wavenumber signal intensity data for the first and second wavenumber intensity data sets by pre-processing the signal intensity data using a pre-processing analytical method, such as a first derivative method, a second derivative method, a standard normal variate (SNV) method, a polynomial fitting method, a multi-polynomial fitting
- 30 method, a mollifier method, a piecewise polynomial fitting (PPF) method or an adaptive iteratively reweighted Penalized Least Squares (airPLS) method;

- (d) obtaining model parameters to be applied to the first and second wavenumber intensity data sets by applying to each one of the pre-processed signal intensity data sets a multivariate regression algorithm, such as a partial least squares (PLS) regression algorithm, optionally wherein the PLS algorithm is a nonlinear iterative partial least squares (NIPALS) regression algorithm or a neural network, wherein a calibration is performed wherein the pre-processed signal intensity data are compared with viral titre data obtained for the same sample conditions using non-Raman spectroscopy methods such as qPCR, RT-qPCR, ELISA or by visual determination by transmission electron microscopy;
- (e) inferring response values using the model parameters obtained from each one of the pre-processed data sets; and
- (f) performing variable selection, optionally variable importance projection (VIP), and identifying Raman spectral variables; and
- (g) optionally performing one or more further rounds of modelling for any of the data sets by re-applying steps (d) to (f) and wherein unimportant variables are removed; and
- wherein the ratio of viral nucleic acids to viruses comprising one or more viral structural molecules in the sample is determined using the model parameters obtained for the identified Raman spectral variables derived from the multivariate data processing models.