

Figs. 18A and 18B include a DRS spectrum from a liquid phantom with 1.8 mg/ml Hb (blue), corresponding model fit (red) and residual (black) and a bar chart showing the Hb concentration curve results, respectively;

5 Figs. 19A and 19B are DRS and IFS spectra, respectively, of phantoms with varying furan concentration (phantom 1 and 2 furan = 0.8 $\mu\text{g/mL}$; phantom 3 and 4 furan = 0.3 $\mu\text{g/mL}$);

Figs. 20A-20E include a diagram of normal and breast cancer tissues placed on glass plate during scanning, a photograph of breast tissues, a composite photomicrograph of histopathology of breast tissues (top insert: ductal carcinoma *in situ*; insert: invasive ductal carcinoma); and DRS and IFS spectra of breast tissues, respectively;

10 Figs. 21A-21H include a DRS spectral intensity map of normal and cancer tissues at 545nm; IFS spectral intensity map at 425nm; DRS parameter maps for scattering parameters A, B and C, Hb and β -carotene, respectively, and an IFS parameter map for collagen;

Fig. 22 is a bar chart of pixel-to-pixel means DRS and IFS parameters in normal and breast cancer tissues;

15 Figs. 23A-23B include a scatterplot of decision algorithm using DRS β -carotene and IFS collagen parameters for selective regions in normal and cancer breast tissues and a diagnostic map of normal and breast cancer tissues using the decision algorithm, respectively, noting that: 1) regions denoted as cancer at the border of the grossly cancerous and normal tissues (white arrows) are an artifact due to inadvertent gaps between the two tissues; and 2) regions denoted as cancer at the outer edges of the tissues (black arrows) are due to the presence of black colloidal ink applied to the margin of the surgical specimen prior to harvesting for use;

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Figs 24A and 24B illustrate reduced scattering and absorption coefficients as a function of wavelength, where the dashed line indicates the raw data and PLS, LUT and LS-SVM model fits are shown with green, blue and red solid lines, respectively. For this representative phantom, PLS (green) and LS-SVM (red) fits for the reduced scattering coefficient are nearly coincident. Similarly, the raw data (black) and LS-SVM (red) fit coalesce for the absorption coefficient plot;

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Figs 25A and 25B are box plots of prediction error percentages for reduced scattering (μ_s) and absorption (μ_a) coefficients, respectively, using PLS, LUT and LS-SVM regression models; the red dotted line indicates the position where the observed values are equal to the reference values in the samples; and

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Fig. 26 is a bar plot of average computation time for the prediction step of PLS, LUT and LS-SVM algorithms.

DETAILED DESCRIPTION OF THE INVENTION

5 An MMS system is generally illustrated in Figure 1A. MMS measurements have been performed on surgical biopsies within 30 minutes of surgical resection. Most of the 30 minute time delay was due to inking and sectioning of the specimen performed as part of the routine pathology consultation performed on these specimens for more information on intra-operative margin assessment in breast cancer patients. IFS, diffuse reflectance and Raman spectra were
10 obtained from a total of 223 spectra from 105 breast tissues from 25 patients. Specimens from patients with pre-operative chemotherapy or who underwent re-excisional biopsy were excluded. DRS and IFS spectra were collected using the FastEEM instrument, followed by collection of Raman spectra with a Raman instrument. Care was taken in placing the Raman probe at the same site on the tissue as the FastEEM probe. Once the spectra were acquired, the
15 exact spot of probe placement was marked with colloidal ink for registration with histopathology. The breast specimens were then fixed and submitted for routine pathology examination, which was performed by a pathologist blinded to the spectroscopy results. The histopathology diagnoses were: 32 normal; 55 fibrocystic change, 9 fibroadenoma and 9 invasive carcinoma (all infiltrating ductal carcinoma).

20 The sampled tissue volume for Raman spectroscopy is 1 mm^3 . Using the combined biochemical and morphologic spectral model, the data are fit to a linear combination of Raman basis spectra for eight breast tissue components: cell cytoplasm, cell nucleus, stromal collagen fibers, fat cells, β -carotene, collagen, calcium hydroxyapatite, calcium oxalate dehydrate, and cholesterol-like lipid deposits (foci of necrosis). The data were then analyzed prospectively
25 using the fit coefficients for stromal collagen (collagen) and fat cells (Fat) in our Raman algorithm for breast cancer diagnosis. A scatter plot and decision lines for the Raman diagnostic algorithm are shown in Figure 1B. A comparison of the Raman spectral diagnoses and histopathology diagnoses is shown in Table 1. The Raman algorithm remained quite robust when applied in a prospective manner to these breast specimens, with an overall accuracy of
30 83%. However, five cases of fibroadenoma were misdiagnosed by Raman as invasive cancer and 4 cases of fibrocystic change were misdiagnosed as cancer.

Pathology \ Raman	Normal	Fibrocystic Change	Fibroadenoma	Invasive Carcinoma
	(32 samples)	(55 samples)	(9 samples)	(9 samples)
Normal	30	7	0	0
Fibrocystic Change	2	41	0	0
Fibroadenoma	0	3	4	1
Invasive Carcinoma	0	4	5	8

Table 1. Comparison of pathologic diagnosis with that of the Raman diagnostic algorithm for *ex-vivo* specimens. The TMS diagnostic algorithm resulted in an overall accuracy of 81% (85/105).

IFS were extracted from the combined fluorescence and DRS. The IFS spectra were analyzed using multivariate curve resolution (MCR) with non-negativity constraints, a standard chemometric method, to extract two spectral components at each excitation wavelength. The resulting MCR-generated spectral components at 340 nm are shown in Figure 2a and Figure 2b, and represent NADH and collagen, respectively, because they are similar to their measured IFS spectra. The spectra are similar, but not identical, as both the lineshape and wavelength maximum of a fluorescence peak obtained from a solution of a pure component is known to be different than that obtained from the same component in a different chemical environment, such as tissue.

For diffusive scattering (μ_s'), wavelength dependence of the form $A\lambda^{-B}$ is used. Two absorbers, oxyhemoglobin and β -carotene, were used to model the extracted absorption coefficient μ_a . Therefore, DRS provided, among other parameters, the amplitude of the scattering coefficient, A , and the concentration of oxyhemoglobin.

The TMS diagnostic method used logistic regression and leave-one-out cross validation, and the analysis was performed in sequential fashion. Scatter plots and decision lines for each step of the diagnostic method are shown in Figure 3a-3c. Normal tissue was identified using the Raman fit coefficients for both collagen and β -carotene (Figure 3a). The finding of low fit coefficients for collagen and β -carotene correlates with histopathology, as normal breast tissue consists largely of adipose tissue, the fat cells which contain large amount of lipid-soluble β -carotene. After the normal tissue was excluded, fibroadenoma was discriminated from fibrocystic change and invasive breast cancer, using the DRS scattering parameter A and IFS NADH fit coefficients (Figure 3b). Fibrocystic change was distinguished from invasive breast cancer using the DRS oxyhemoglobin and IFS collagen fit coefficients at 340 nm (Figure 3c).

This diagnostic method uses contributions from both the cells (NADH) and the stroma (collagen). However, it is unclear why the fit coefficient for collagen and scattering parameter A should be lower for fibroadenoma than for invasive carcinoma and fibrocystic disease, or the fit coefficients for oxyhemoglobin should be higher for invasive breast cancer than for fibrocystic disease. A comparison of the TMS spectral diagnoses and histopathology diagnoses is shown in Table 2. The overall accuracy (correct prediction of each of the pathologies) is 87.6% (92/105). Although the overall accuracy of the two techniques is comparable in this small data set, all of the invasive carcinomas were diagnosed correctly by TMS and only 4 normals or fibrocystic changes were misclassified as invasive carcinoma.

Pathology \ TMS	Normal	Fibrocystic Change	Fibroadenoma	Invasive Carcinoma
	(32 samples)	(55 samples)	(9 samples)	(9 samples)
Normal	27	7	0	0
Fibrocystic Change	2	47	0	0
Fibroadenoma	0	0	9	0
Invasive Carcinoma	3	1	0	9

Table 2. Comparison of TMS and histopathologic diagnosis for ex vivo study of fresh surgical breast biopsies. The TMS diagnostic algorithm had an overall accuracy of 87.6% (92/105).

The measurements were obtained using TMS and Raman spectroscopic techniques independently, but they can also be obtained using a combined diagnostic procedure. In developing the MMS algorithm, only parameters that were diagnostic in one of the three individual spectroscopic modalities were used. The diagnostic parameters from TMS are scattering parameter A, and the fit coefficient for oxyhemoglobin, β -carotene, and NADH and collagen by IFS at 340 nm excitation wavelength. The diagnostic Raman parameters are the fit coefficients for fat and collagen. Like the TMS diagnostic procedure, this algorithm incorporates contributions from both the epithelial cells (NADH) and stroma (collagen).

The MMS diagnostic method was developed using logistic regression and leave-one-out cross validation. As with TMS, the analysis is performed in sequential fashion. Figs. 4A-4C display the scatter plots and decision lines for each of the three steps performed in the MMS diagnostic algorithm. First, normal tissue was identified using the Raman fit coefficient for collagen. This is the only change in this algorithm than that used for TMS, where the first step was identification of normal tissues using the intrinsic fluorescence fit coefficient for collagen at 340nm (Figure 4A). The next two steps are identical to those in the TMS diagnostic algorithm,

with fibroadenoma distinguished from fibrocystic change and invasive carcinoma using scattering parameter A and the fit coefficient for NADH (Figure 4b), and fibrocystic disease distinguished from invasive breast cancer using the fit coefficients for oxy-hemoglobin (Figure 4C). A comparison of the MMS spectral diagnoses and histopathology diagnoses is shown in Table 3. The overall accuracy is 92% (92/105), and is only slightly improved for MMS as compared to TMS. As with TMS, all 9 invasive carcinomas were diagnosed correctly by MMS. But this time, only 2 fibrocystic changes and no fibroadenoma are diagnosed as invasive carcinoma.

Pathology Multimodal	Normal	Fibrocystic Change	Fibroadenoma	Invasive Carcinoma
	(32 samples)	(55 samples)	(9 samples)	(9 samples)
Normal	30	4	0	0
Fibrocystic Change	2	49	0	0
Fibroadenoma	0	0	9	0
Invasive Carcinoma	0	2	0	9

Table 3. Comparison of MMS and histopathologic diagnosis for the ex vivo study of surgical breast biopsies. The MMS diagnostic algorithm had an overall accuracy of 92.4%.

Table 4 shows a detailed comparison of the diagnostic performance of all three methods, Raman, TMS and MMS, with MMS providing the best sensitivity and specificity, as well as overall accuracy. By introducing a parameter from the Raman model to the first step a greater number of correctly diagnosed normal tissues. Figure 4A is a box plot, which illustrates the average values (red line), the interquartile range (blue box), and outliers (red plusses), of collagen content for each pathology. Previously, the collagen content from TMS was analyzed in this manner but did not show the same success. Although both Raman and TMS (and thus MMS) are sensitive to collagen, each uses a different wavelength of light (Raman at 830 nm and TMS at 340 nm). Therefore, their sampling volumes are different. This fact explains why collagen fit coefficients extracted via Raman spectroscopy do not strongly correlate with collagen fit coefficients extracted using TMS. This is likely because of the different sample volumes (depths) of the TMS and Raman modalities. With a smaller sampling volume, TMS did appear to sample deep enough into the tissue to assess collagen adequately.

The results indicate that MMS, a combination of DRS, IFS, and Raman spectroscopy provides better results than those obtained from each technique alone. This can result from

the combined MMS diagnostic algorithm combines spectral parameters derived from both epithelial cells and stroma and (taken together) have a larger sample volume.

Modality Performance	Raman	TMS	MMS
Sensitivity	89%	100%	100%
Specificity	91%	96%	98%
Overall Accuracy	81%	88%	92%

Table 4. Comparison of performance of Raman, TMS and MMS algorithms for the diagnosis of breast cancer.

As in breast cancer, the development of atherosclerosis is governed by subtle chemical and morphological changes in the arterial wall, manifesting themselves in the development of a plaque that causes luminal obstruction. Many of these changes are the result of metabolically active inflammatory and smooth muscle cells, such as foam cells, that degrade LDL and release it into the necrotic core in the form of ceroid and other LDL degradation byproducts.

The preferred method for the diagnosis of atherosclerosis are based on a linear combination model that yields fit coefficients for 10 morphological components of artery wall, including collagen fibers (CF), elastic lamina (EL), smooth muscle cells (SMC), adventitial adipocytes (AA), cholesterol crystals (CC), β -carotene crystals (β -CC), foam cells/necrotic core (FC/NC) and calcium mineralizations (CM). A preferred algorithm was developed for classification of lesions as non-atherosclerotic, non-calcified plaque and calcified plaque. This diagnostic algorithm was based on combined fit coefficients for cholesterol crystals + foam cells/necrotic core (the latter two having indistinguishable Raman basis spectra) and the fit coefficient for calcium mineralizations.

A preferred embodiment relates to a procedure for measuring vulnerable plaque. These are most often plaques with a thin fibrous cap overlying a large necrotic lipid core, and may have other features of vulnerability including foam cells and other inflammatory cells, intraplaque hemorrhage or thrombosis. A second Raman algorithm capable of diagnosing vulnerable plaque with about the same sensitivity and specificity as a previous algorithm for plaque classification (~85-95%). This second algorithm for the diagnosis of vulnerable

plaque makes use of the fit coefficients of 5 artery morphological components: the combined fit coefficients for foam cells + necrotic core and the fit coefficient for calcifications, as in the previous algorithm, plus the fit coefficients for collagen and hemoglobin. A preferred algorithm for the diagnosis of vulnerable plaque involves using spectral parameters that distinguish between metabolically active foam cells and the non-metabolically active necrotic core.

Fluorescence, reflectance and LSS provide information about cell metabolism and tissue scatterers such as foam cells, the cytoplasm of which is filled with a foam-like aggregate of lipid-filled lysosomal vesicles where the metabolism and degradation of LDL takes place. Therefore, by combining Raman spectroscopy with fluorescence, reflectance and optionally LSS, an algorithm for the diagnosis of vulnerable plaque incorporates contributions from metabolically active, potential scatterers like foam cells as well as non-metabolically active plaque constituents like the necrotic core. But, MMS has a further advantage for the diagnosis of vulnerable plaque, and that is the ability to provide depth information about key biochemical and morphologic structures like the fibrous cap, that too undergoes degradation, this time, by matrix metalloproteinase that renders it more prone to rupture.

In vitro measurements of MMS for the diagnosis of vulnerable plaque using 17 frozen archival tissues from carotid endarterectomies have been performed.

TMS spectra were collected using the FastEEM instrument and Raman using the clinical Raman system, with the associated probes. Care was taken in placing the Raman probe at the same site on the tissue as the FastEEM probe. Once the spectra were acquired, the exact spot of probe placement was marked with colloidal ink for registration with histopathology. The artery specimens were then fixed and submitted for routine pathology examination, which was performed by a cardiovascular pathologist blinded to the spectroscopy results. The histopathology examination of the lesions included an assessment of a number of histologic features of vulnerable plaque, including thickness of the fibrous cap, size of the necrotic core, superficial foam cells, intraplaque hemorrhage and ulceration. The histopathology results are summarized in Table 5. A vulnerable plaque index (VPI) was assigned to each specimen. Of the 17 lesions, 4 exhibited VPI scores ≥ 10 and were classified as vulnerable plaques.

MMS spectral analysis for artery was similar to that for the breast. Again, OLS is used to fit the Raman data using the morphological model. The DRS spectra were fit using the diffusion theory model. Modeling of the DRS spectra yielded, among other parameters,

scattering coefficient A and hemoglobin concentration. IFS were analyzed using MCR with non-negativity constraints to find two spectral components at 308 nm and 340 nm. The IFS data was fit using ordinary least squares (OLS) using the two MCR components as the model. The Raman basis spectra, DRS extinctions and IFS MCR components are shown in Figures 5A and 5B.

	SNOMed Class.	VPI	Intimal or Fibrous cap Thickness (microns)	Necrotic Core Thickness (microns)	Foam Cell Depth (microns)	Foam Cell Grade (0-3+)	Intraplaque Hemorrhage	Ulceration
1	IF	5	24-64	NA	NA	NA	NA	NA
2	IF	5	40-80	NA	NA	NA	NA	NA
3	ATS	0	480-500	NA	480	3+	NA	NA
4	ATS	5	240-440	NA	40	1+	NA	NA
5	ATS	0	456-536	NA	456	2+	NA	NA
6	ATM	5	200-320	400	280	2+	NA	NA
7	ATM	5	460-640	560	NA	NA	NA	NA
8	ATM	5	440-500	4800	440	2+	NA	NA
9	ATM	5	1000-1500	6400	1800	1+	NA	NA
10	ATM	5	520-640	1340	640	2+	NA	NA
11	CATM	6	140-160	1640	88	1+	NA	NA
12	CATM	7	120-480	4000	120	1+	NA	NA
13	CATM	5	1440-1500	240	280	1+	NA	NA
14	FS	6	2-160	16	NA	NA	Acute	Plaque Disruption
15	FS	13	40-80	NA	115	NA	NA	NA
16	ATS	10	22-50	125	0	1+	NA	NA
17	ATM	5	640	1600	20	2+	NA	NA

Table 5. Morphological characteristics of the 17 specimens. IF = infimal fibroplasias, ATS= atherosclerotic, ATM=atheromatous, FS=fibrotic-sclerotic, C=calcified.

Figure 6A-6C shows the spectroscopic data and model fits for three different artery lesions, an intimal fibroplasia (A), a non-vulnerable plaque (B) and a vulnerable plaque (C). All of the MMS spectra can be fit very well using the previously described models.

Four spectral parameters were correlated with the histopathologic features of vulnerable plaque: DRS scattering parameter A and hemoglobin concentration; an IFS parameter $\rho = C_{308}/C_{340}$, where C_{308} and C_{340} are the contributions of the more blue-shifted MCR basis spectra; and the Raman parameter $\Sigma = CC + FC/NC$, where CC and FC/NC are the relative contributions in the Raman spectra of cholesterol crystals and foam cells + necrotic core, respectively. The diagnostic potential as it relates to assessing plaque vulnerability for each of the spectral parameters will be discussed separately in the next paragraphs.

As described earlier, intraplaque hemorrhage is a marker of plaque vulnerability. Histopathology indicates that the lesion in specimen #14 is the site of acute intraplaque

hemorrhage (Table 5); whereas the other lesions not hemorrhagic. Fig. 7 displays the hemoglobin concentration fit parameters of the 17 specimens obtained from the DRS spectra. The lesion in specimen #14 exhibits a distinctly high c_{Hb} , and a threshold value of $c_{Hb} = 5$ separates it from the remaining lesions. This suggests that the concentration of hemoglobin inside the arterial wall, measured with DRS to sense acute intraplaque hemorrhage.

Superficial foam cells are important in assessing plaque vulnerability as they are often present in the fibrous cap near plaque erosions and ruptures, and are a likely source of MMPs that degrade the fibrous cap and lead to plaque rupture. Figure 8 displays the DRS scattering parameter A (relative units) for the 17 specimens. Foam cells are present in all 10 lesions with $A > 2$, where they occur at an average depth of 250 microns below the intimal surface of the plaque (Table 5). In contrast, foam cells are observed in only 2 of the 7 lesions with $A < 2$, and these foam cells tend to reside deeper in the plaque, at an average depth of 1100 microns (Table 5). Given the several hundred micron penetration depth of DRS at visible wavelengths, DRS does not sense such deep foam cells, which are not clinically relevant to plaque vulnerability. Hence the scattering parameter A appears to be a measure of the presence of superficial foam cells. The correlation of A with foam superficial suggests that the presence of foam cells near the tissue surface can markedly enhance scattering, and that foam cells, which contain a high concentration of lysosomal vesicles, are strong light scatterers. In addition this data suggests that, using parameter A , the method differentiates the presence of foam cells from that of necrotic core, which Raman spectroscopy alone cannot do.

As discussed above, an important feature of vulnerable plaque is the presence of a thin fibrous cap. A cap thickness of less than 65 μm is an established criterion of vulnerability. IFS spectra at 308 and 340nm excitation wavelengths were obtained to parameterize fibrous cap thickness. Two MCR spectral components to be associated with collagen and/or elastin, structural proteins that characterize the upper layers of both normal artery (normal intima) and atherosclerotic lesions (fibrous cap). Comparing the MCR spectra to the known spectral of those fluorophores, the red-shifted spectrum resembled elastin while the blue-shifted spectrum is similar to collagen (Fig. 5). The corresponding fit coefficients, C_{340} and C_{308} , relate to the amount of collagen present within the tissue volume sampled. The sampling depth with 340 nm excitation ($\sim 60 \mu\text{m}$) is greater than that with 308 nm excitation ($\sim 50 \mu\text{m}$). Thus, C_{340} provides information about collagen and elastin distributed over a much greater depth compared to that provided by C_{308} . Hence, the ratio $\rho = C_{308}/C_{340}$ can provide

information about the thickness of the fibrous cap. Fig. 9 plots ρ for the 17 specimens. Lesions with the highest values ($\rho > 2$, specimens #1 and 14-16) have the lowest average intimal or fibrous cap thicknesses, all below 50 μm . Conversely, for each of the remaining specimens, for which $\rho < 2$, the average cap thickness is greater than 50 μm . The one exception to this is Specimen #17, an ulcerated plaque, which has a variable fibrous cap thickness, ranging from 0 to 200 μm , and yet it has a $\rho < 2$. Nevertheless, these results indicate that a threshold value $\rho = 2$ can be used to identify thin fibrous caps.

For Raman spectroscopy, the parameter $\Sigma = \text{CC} + \text{FC}/\text{NC}$ is an indicator of the presence of necrotic material, foam cells and cholesterol crystals. The values of Σ for the 17 carotid artery specimens are plotted in Fig. 10. Specimens rich in foam cells or necrotic core exhibit larger values of Σ . A threshold value of $\Sigma = 40$ separates specimens of low and high overall lipid content. The only exceptions are specimens #14 and #15, which have high values of Σ although histopathology indicates the absence of foam cells and/or necrotic core. These two specimens are fibrotic-sclerotic plaques. They are morphologically unusual, demonstrating a well-developed fibrous cap but lacking an extracellular necrotic core and cholesterol crystals. These can be viewed as end stage plaques.

The key spectroscopic parameters obtained from IFS, DRS and Raman spectroscopies are displayed together in Table 6 for all 17 specimens. This method uses yes/no results based on the threshold values rather than numerical values. Each column represents a spectroscopic marker of a histologic feature of vulnerable plaque: Hb, indicative of intraplaque hemorrhage; scattering parameter A, indicative of foam cells close to the surface; ρ , indicative of fibrous cap thickness; and Σ , indicative of the build up of necrotic core material. Note that 3 of the 4 vulnerable plaques can be identified by detecting a thin cap ($\rho > 2$) together with another parameter such as A or Σ .

The ability of MMS to provide depth-sensitive information is more relevant to measurements of atherosclerosis than those of breast cancer because of the layered structure of the arterial wall. Define the optical penetration depth as the depth at which the power of light incident on a tissue sample falls to $1/e$ of its incident value. Generally the optical properties of aorta indicates penetration depths of about 90, 150 and 1200 microns for light of wavelengths 308, 340 and 830 nm, respectively. The penetration depths at different IFS wavelengths were measured by incrementally stacking 20 μm thick sections of aortic media. The FastEEM probe tip was placed in contact with the tissue and the transmitted power was measured as a function of tissue thickness. The penetration depths at 308 and 340 nm were measured as 85 and 105

μm , respectively. These values correspond with prior results especially noting the variability of human tissue. They also agree with estimates obtained from the formula $\delta = 1/\mu_{\text{eff}} = 1/\sqrt{3\mu_a(\mu_a + \mu_s)}$, using the known scattering and absorption properties of arterial tissue at different wavelengths; Fig. 11A gives the μ_a and μ_s in the visible wavelength range.

5 Note that in the single-ended geometry of our artery measurements (i.e. the probe both delivers and collects light at the same position) the sampling depth, can be defined as $1/\delta_s = 1/\delta_{\text{ex}} + 1/\delta_{\text{em}}$, where δ_{ex} and δ_{em} are the penetration depths of the excitation and emission light, respectively. The sampling depth characterizes the attenuation of both the excitation and the emitted light, which can be at a longer wavelength, as in the case of
10 fluorescence or Raman scattering. Thus the sampling depths of IFS₃₀₈ and IFS₃₄₀ are much smaller: 50 and 60 μm , respectively, taking into account the longer wavelengths of the emitted light. A previous measurement established a sampling depth of 470 μm for Raman spectroscopy of artery using 830 nm excitation. In the following, use 50, 60 and 470 μm as the sampling depths at 308, 340 and 830 nm, respectively. Note that the definition of penetration as the length
15 where light is attenuated to 1/e of its original value is somewhat arbitrary and that, optionally the device can sample deeper than those values. Similarly, different wavelength regions of the diffuse reflectance spectra sample tissue at different depths. In general, short wavelength IFS (308 nm, in particular) provides information about the top layer (intima/fibrous cap), longer wavelength IFS samples somewhat deeper, and Raman spectroscopy has the greatest sampling
20 depth. Fig. 12 gives the sampling depths at various wavelengths in the range 308-830 nm, comparing values from our experimental studies those calculated from the literature (the emission wavelength is chosen to be the same as the excitation so $\delta_s = \delta_{\text{ex}}/2$).

Multimodal spectroscopy (MMS) is a spectral diagnosis technology that combines spectroscopic results from TMS and Raman spectroscopy to provide more accurate disease
25 diagnosis and a more comprehensive picture of biochemical, morphological and metabolic state of the tissue as it relates to disease pathogenesis and pathophysiology. Figs. 11B-D illustrate in vivo Raman (Fig. 11B) diffuse reflectance (Fig. 11C) and intrinsic fluorescence (Fig. 11D) spectra taken of a femoral artery. The artifact between 600 and 700 nm in the IFS spectrum is due to the surgical light in the room which can be turned off during use.

30 The results have demonstrated that combining information from Raman, fluorescence and reflectance spectroscopies provides better diagnostic accuracy than that provided by any one of the spectroscopic techniques independently, and that differences in sampling volumes can be used to advantage for depth sensing.

The present invention relates spectroscopic diagnosis of a wide range of diseases including oral, esophageal, colon and cervical cancer, as well as the diagnosis of vulnerable atherosclerotic plaque and breast cancer. A preferred embodiment spectroscopically extracts biochemical, morphologic and metabolic information related to features of plaque vulnerability or predictive of breast cancer. More than rendering precise disease diagnoses, the system extracts accurate biochemical, morphologic and metabolic information about tissue composition. The system stores IFS morphological basis spectra using microspectroscopy, and performs ex vivo and in vivo tissue measurements using DRS, IFS, and Raman spectroscopic techniques.

Combined MMS spectral data provides insight into depth dependent morphological features of breast cancer (collagen) and vulnerable plaque (fibrous cap thickness and superficiality of foam cells). These measurements simultaneously collect and analyze Raman, DRS and IFS spectra from the same spot without registration errors using an MMS probe.

Quantitative information about biochemical and morphological tissue components are provided from DRS and Raman spectra using basis spectra in our linear combination model. IFS can also provide quantitative information. Meaningful data modeling can be obtained using fluorescence basis spectra measured from biochemical and morphologic tissues structures measured *in situ* uses the IFS technique to remove the artifacts of tissue absorption and scattering. This can be useful as basis spectra obtained by microspectroscopy of thin ($< 6 \mu\text{m}$) tissue sections or cell cultures can have little or no scattering or absorption effects, and thus may not model uncorrected raw fluorescence spectra as well as IFS spectra.

To build representative libraries of basis spectra, 50-100 spectra were acquired each from a variety of tissue and cellular sources. Tissue handling and preparation methods can lead to spectral distortions. For example, increased absorption has been observed in frozen-thawed tissue, possibly the result of red blood cell lysis, with a concomitant decrease in tissue fluorescence. These changes are less significant in artery wall than in epithelial tissues. Several steps are taken to minimize these artifacts in the collection of IFS basis spectra. First, all IFS basis spectra are collected from freshly excised tissues within 30-60 minutes of excision.

In the case of artery, basis spectra are obtained initially from cryostat sections of fresh tissue that has been immediately snap frozen in liquid nitrogen. Basis spectra are obtained on these sections within minutes of preparation. The passively thawed frozen sections maintained in a humid chamber to prevent drying.

Optionally, basis spectra obtained either from fresh tissue sections (or short term organ cultures) maintained in a balanced electrolyte solution such as Hanks Balanced Salt solution at

neutral pH. Under these conditions it is known that tissue remains viable for at least 90 minutes, with minimal changes in fluorescence. Basis spectra can also be obtained from live human cell cultures, where appropriate, to provide a relatively pure population of cells. Cell cultures from which basis spectra may be obtained for artery studies include primary cultures of normal human endothelial and smooth muscle cells and various cell culture models of foam cells, such as LDL fed human alveolar macrophages. Cell cultures from which basis spectra may be obtained for the breast studies include primary cell cultures of normal breast epithelial cells, myoepithelial cells and fibroblasts and human breast cancer cell lines.

The basis spectra can be collected using a confocal microscope adapted for TMS microspectroscopy. A confocal fluorescence system uses excitation light generated by the FastEEM instrument. The excitation light from the FastEEM is delivered from a 200 um fiber, focused to 100 um aperture and collimated. The collimated light is delivered down to the objective using a neutral density beam splitter (90/10) and collected light from the thin tissue is focused to a confocal pinhole. This light is delivered to the FastEEM spectrograph and CCD via optical fibers. The microscope stage is programmed to FastEEM scan in the features of interest. A bright field image of the specimen is obtained and used for registration between pathology and spectroscopy. The FastEEM software is synchronized for operation between the microscope and FastEEM excitation source and CCD camera.

With the library of biochemical and morphological basis spectra morphological basis spectra (of such structures as foam cells in atherosclerosis and epithelial cell nuclei and cytoplasm in breast cancer) are fit with the same linear combination method used previously for Raman spectroscopy, using biochemical basis spectra to determine their precise chemical composition and identify the fluorophores characteristic of each structure. The basis spectra are also fit to *ex vivo* IFS tissue spectra, and quantitative information about the presence of fluorophores (tryptophan, collagen, elastin, NADH, FAD, β -carotene) and the morphologic structures they comprise, is extracted. Using this quantitative spectral information obtained from all three spectral modalities, an automated method to characterize morphological components associated with disease state, including their depth profiles, is provided. Quantization of the biochemical and morphologic composition of the tissues is incorporated into algorithms for the diagnosis of vulnerable plaque and breast cancer. Similar basis spectra libraries, spectral models and diagnostic algorithms are used for cancers of the oral cavity, colon, bladder and cervix.

Using at least 200 spectra each from *ex vivo* fresh arterial (carotid and femoral) and breast tissues from at least 40 different patients spectra are acquired using the MMS instrument

using the integrated MMS probe. The location of the spectroscopic site is established by attaching a metal sleeve to the probe that can make a shallow incision around the site. After removing the probe, the location can be marked with an ink dot. The sample can be fixed in formalin and submitted for histopathological examination, by a pathologist. Both spectral analysis and quantitative image analysis (QIA) of the samples is performed in parallel, using the same tissue site for both measurements.

To evaluate the depth sensing capabilities of different fluorescence excitation wavelengths, Monte Carlo models are employed to simulate light propagation within tissue. Monte Carlo models can have simple layered structures with physiological dimensions and optical properties to simulate light propagation in the normal arterial or breast tissue. Optical properties can be measured with an integrating sphere. The spatial distribution of morphological features associated with vulnerable plaque or breast cancer are estimated using QIA software. This information, along with the IFS basis spectra, are used as input into fluorescence Monte Carlo models to evaluate the ability of different excitation wavelengths to probe morphological structures such as foam cells and necrotic core.

DRS provides information about the presence of Hb, indicative of thrombus or intraplaque hemorrhage, and the amplitude of the scattering coefficient A is related to the presence of foam cells and their depth within the artery wall (superficiality). IFS provides information about fibrous cap thickness through the ratio of MCR components at 340 to 308 nm excitation. Raman spectroscopy also provides information related to the presence of foam cells or necrotic core. Thus MMS modalities provide important diagnostic parameters related to collagen (Raman and IFS), diffusive scattering (DRS) and NADH (IFS) that are of use for breast cancer diagnosis.

There are additional correlations between IFS and DRS-measured parameters and key morphological features of breast cancer and vulnerable plaque. For example, detection of β -carotene by DRS can be a strong marker of tissue necrosis and extracellular lipid pools. Tryptophan is another fluorophore that plays an important diagnostic role in both atherosclerosis and breast cancer.

Fit coefficients from MMS morphological models can be used to predict disease/tissue parameters using logistic regression. These fit coefficients can be used as parameters for an algorithm for distinguishing vulnerable and non-vulnerable plaque and the full spectrum of breast lesions, both benign and malignant.

Spectroscopic instrumentation for MMS can comprise a combined instrument in which a clinical Raman system and a FastEEM are linked together for use with a single combined

spectral probe. Alternatively a smaller integrated clinical instrument for a variety of clinical studies involving atherosclerosis, breast cancer Barrett's esophagus and oral cancer. A number of specialized MMS spectral probes can be used for front-view, side-viewing and circumferential imaging modes. See for example U.S. Application No. 10/407,923 filed on 5 April 4, 2003, the entire contents of which is incorporated herein by reference. The measurement for breast cancer and atherosclerosis can be obtained using two independent instruments and separate spectral probes. Due to the differences in these probes, which determine the light collection efficiency, it is preferable to use a single probe. This will eliminate registration uncertainties between Raman and DRS/IFS data and ensure that 10 illumination areas will be the same. This instrument provides the full, range of fluorescence excitation wavelengths and can include a front-looking MMS spectral probe.

A combined instrument can use a FastEEM (See U.S. Patent No. 6,912,412 incorporated herein by reference) and Raman system combined under a single LabView software program that synchronizes the operation of both units. This instrument collects a set of IFS spectra and a 15 DRS spectrum in 0.2 seconds, followed by collection of a Raman spectrum in 1 second, for example. Excitation light from FastEEM and Raman sources is coupled into a single tapered fiber with 0.22 NA. The tapered fiber has a 600 μm core diameter at one end allowing up to four excitation inputs and can be drawn down to a single 200 μm core for use at the distal end of the probe. For ease of fabrication, MMS probes can be assembled with 15 collection fibers 20 surrounding the central excitation fiber. Alternatively a reduced diameter device has 9 fibers around a single fiber in the probe. The 15 fibers are split at the proximal end so that 10 of the 15 fibers are coupled into the Raman spectrograph while the remaining 5 fibers are coupled to the FastEEM spectrograph. The collection fibers have a core diameter of 200 μm with 0.26 NA. High NA Anhydroguide G fibers can be used in the Raman instrument. They are well suited for 25 near IR wavelengths but have a 40-50% transmission loss in the 300-400nm region. The Superguide G fibers used in FastEEM have negligible transmission losses in the same UV wavelength range, but low NA. In spite of transmission losses in Anhydroguide G fibers, the spectral quality is not significantly reduced, due to the strength of the fluorescence and reflectance signals at these wavelengths. In one embodiment of an MMS probe, both 30 Superguide and Anhydroguide fibers are used in a single probe to provide a baseline performance level with the optimum transmission properties.

Of the three spectral signals (Raman, DRS and fluorescence), Raman is typically the weakest. Thus, a spectral probe capable of collecting high-quality Raman spectra should easily

collect fluorescence and reflectance spectra as well. The spectral probe design for the combined instrument is single-ring front-viewing Raman probe.

5 Placement of filters and ball lens can be the same as the Raman probe, but the filter characteristics has tighter specifications when used with all three spectral modalities. Figure 13A illustrates the details for a reduced diameter 9-around-1 probe 100 and excitation/collection trajectories through a ball lens 106 that contacts tissue 108. Similar to the Raman probes, the filter module has a filter rod 104 placed on the delivery fiber with transmittance from 300-830 nm and no transmittance (<1%) beyond 850nm. A filter tube placed on the collection fibers has transmittance from 300-810 nm and from 850-1000 nm and with a narrow 40 nm band centered at 830 nm having low transmittance. An end view of the probe is shown in Fig. 13B with collection fibers 112 positioned in a circular array around central excitation fiber 102. A side looking probe 120 is shown in Fig. 13C in which a half ball lens 130 is in contact with a mirror 132 to reflect light from excitation fiber 124 and filter rod 128 through sapphire window 134. Light returning from the tissue such as artery wall is reflected into collection fibers 122 through long pass filter tube 127. A metal sleeve 125 surrounds filter 128. An aluminum jacket surrounds the excitation fiber 126. A Teflon jacket 135 provides the cylindrical tube that forms the outer wall of the catheter.

15 In Fig. 13D an end view of a design in which a first group of 3 collection fibers 140 are used to collect reflected light and 3 pairs of fibers 144 collect the Raman light passing through ball lens 160. The central fiber 142 directs light through the forward looking probe with lens 160 in Fig. 13E or half ball lens 170 of Fig. 13F. The filter system used in the probe is shown in Fig. 13G.

20 The wavelength-dependent sampling volume and depth of penetration of the probe can be determined with tissue phantoms and/or thin sections of tissue. The diameter of the excitation spot illuminating the tissue can be approximately equal for all wavelengths; however, the tissue penetration depth is different for different excitation wavelengths. Because the spot diameter and penetration depth are important for diagnostic algorithms and they are measured and checked with Zemax optical design models and Monte Carlo models.

25 A compact portable MMS instrument that incorporates all three spectroscopic modalities (DRS, IFS and Raman) is shown in Fig. 14A. The fourth modality, LSS, requires no extra instrumentation. A preferred MMS instrument 200 uses solid state light emitting diodes, reducing the instrument size, complexity and cost, and eliminate many maintenance issues related to excimer laser and dye cell operation. The MMS instrument can employ a common spectrograph 202 and CCD 204 for all spectral acquisition. To accommodate the requirements

for using all three spectroscopic modalities, spectra are collected over the wavelength range 300-1000nm. Excitation light for each modality is delivered sequentially to the sample, and fluorescence, DRS and Raman spectra are acquired. This is followed by real-time analysis of the data, during which IFS spectra are derived from the fluorescence and DRS spectra. The information from the different modalities provides depth-sensitive, complementary chemical and morphological information on tissue sites.

The measurements include IFS spectra excited at 308 and 340 nm, DRS and Raman spectra. The combined TMS/Raman instrument is used for FastEEM fluorescence excitation wavelengths to determine the diagnostic value of the various excitation wavelengths. The most appropriate two or three fluorescence wavelengths can be used in the integrated system.

Data acquisition, analysis and tissue characterization preferably occurs in 5 sec or less. Triggering of the light sources is accomplished by means of a National Instruments Timer/Counter card and a Princeton Instruments CCD controller, respectively. The sequence of operation can be controlled by computer 205 as follows: (1) Initialize CCD for spectral acquisition; (2) open shutter for the CCD and activate insertion of appropriate collection filter; (3) trigger light source (LED, diode laser or flashlamp); (4) acquire spectrum; (5) close shutter; (6) read/transfer data and store in computer 206 and display at 208. The time for acquiring all spectra depends upon the excitation power, thus the exposure time can be adjusted to accommodate signal levels.

Separate excitation and reflectance sources can be used for each spectroscopic modality. Laser emitting diodes 214 (~1 mW) provide fluorescence excitation light at 308 and 340 nm, a 60W xenon flashlamp generates a continuous spectrum from 300-1000 nm for DRS, and a laser diode 212 at 830 nm (500 mW) will generate the Raman excitation light. A flashlamp 218 can be used in the FastEEM, and the 830 nm laser diode in the Raman system. Each of these four light sources can be focused onto separate 200 μ m core diameter optical fibers, and then coupled together into a 600-to-200 μ m tapered optical fiber. The output can be connected to the combined spectral probe via an SMA connector. The system enables fluorescence excitation wavelengths to be added and/or changed.

UV diode sources can be used compact light sources in the 300-340 nm range available. UV light emitting diodes at wavelengths as short as 275 nm or UV LEDs in the 305-360 nm wavelength range can be used. Present 308 nm LEDs produce 1-2 mW of CW power in a bandwidth of 10-15 nm, emitted from a 0.1 mm aperture over a 30° angular range. Because of this large bandwidth, a filter can be used to restrict the light to a 2 nm bandwidth. Thus, under

present conditions, $\sim 1 \mu\text{J}$ of 308 nm light can be delivered via 200 micron core, 0.26 NA, fused silica optical fiber in 10 ms, resulting in the acquisition of high SNR fluorescence spectra. Characteristics of 340nm LEDs are even more favorable.

Each of the spectral probe collection fibers, typically nine, (fifteen in one design) are coupled to an SMA connector mounted on the front panel of the instrument. Long (wavelength) pass filters 220 mounted on a programmable wheel driven by a stepper motor are positioned in the return beam path to prevent Raman and fluorescence excitation light scattered from the tissue from entering the spectrograph. Since the reflectance measurements cover a broad range (300-1000 nm), the acquired spectra contain second order contributions. Taking two reflectance measurements, one with no filter and another with a long pass 500 nm cutoff filter (mounted on the wheel), eliminates these contributions. The unfiltered reflectance provides spectral information below 600 nm, and the filtered reflectance provides information above 500 nm. The Princeton Instruments Spec10:400BR CCD camera of the Raman system can be coupled to an Acton Research Spectra Pro 150 spectrograph with a grating blazed at 500nm and 200 grooves/mm. Alternatively two separate gratings or dispersive elements can deliver different light modalities onto separate regions of the detector.

This combination of fluorescence, reflectance and Raman capabilities in one instrument provides a compact clinical instrument. With a single spectrograph/CCD combination, a spectral range of 300-1000 nm is covered, compared to 155 nm in our existing Raman system. This causes an increase in spectral dispersion by a factor of 4.5, and a reduction in system resolution from 10 to 45 cm^{-1} . However, if the spectral resolution degrades the accuracy of the Raman fit coefficients significantly such that diagnostic accuracy is also degraded. A two spectrograph/CCD system can also be used with one spectrograph/CCD combination is dedicated to Raman while the other to fluorescence/reflectance. A high-speed mirror will direct the collected light to appropriate spectrograph/CCD combination.

A further embodiment of a system 250 is shown in Fig. 14B in which a translational stage 270 is used to couple light from the source sequentially into the probe 252. This contrasts with the prior embodiment where the sources are coupled to probe 240 with combiner 230 to provide simultaneous illumination. The delivery 244 and collection 242 filters are shown schematically. Another source 260 is also used and accounted for in the filter which 284, spectrograph 280 and detector 282 system.

The detection of vulnerable plaques, margin assessment in breast cancer and transdermal needle biopsies can be performed using front-viewing, side-viewing or circumferential imaging probes.

Using the integrated MMS system, spectra are collected from several of these margins prior to excision and thus only tissue that would normally be excised during the procedure will be removed. During each procedure, the distal end of the sterilized MMS front-viewing probe is placed in gentle contact with the marginal breast tissue in the surgical cavity under direct visualization while spectra are acquired. All room and surgical lights will be turned off during the measurements. The spectrally examined marginal tissue will then be excised by the surgeon and submitted for conventional pathological examination.

Under local anesthesia following a manual incision of the skin, a cannula having a diameter 0.5 to 2 cm is advanced toward the suspect lesion guided under ultrasound or stereotactic mammography. The central channel of the needle contains a circular blade that is used to cut the biopsy and will provide access for the MMS probe. Once positioned in the lesion, a MMS side-viewing probe is inserted in the central channel and acquires a series of spectra as the probe is withdrawn along the opening. The probe is withdrawn and cutting blade replaced and a biopsy is acquired. Biopsies are performed over a 360 degree around the axis of the needle without it being withdrawn with typically twelve cores of tissue removed using 11 to 14 gauge needles. The excised biopsy specimens are submitted for specimen radiography to document the presence of calcification and then conventional pathology.

A digital photograph of the lesion and probe placement is recorded. Precise registration between the probe location and biopsy site is ensured by immediately scoring a circular region of tissue slightly larger than diameter of the probe with a 1.5 mm punch biopsy. A larger punch biopsy (~3.5 mm) is used to remove a larger tissue specimen for histopathology and slide preparation. The smaller mark is located later when viewing the slide under the microscope.

A portable tissue scanner was constructed that can scan large tissue specimens (up to 20 cm x 20 cm or larger, for example) at high resolution (at least 0.25 mm) in a clinically acceptable time frame (less than 20 minutes). Fig. 15A shows a schematic diagram of a tissue scanner system 400 in accordance with the invention. The scanner employs a unitary multimodal optical fiber probe 410, or probes, that can be employed in a multimodal clinical spectroscopy system for point spectroscopy measurements. Two (or three) optical fiber probes can also be used, one for DRS, another for IFS (and optionally, a third for Raman measurements), at a fixed separation of 0.75cm to minimize cross talk between the probes. For the single probe system, a probe shown in Fig. 15B using a single light delivery fiber 142, and pairs of separate collection fibers 140, 144 and 146 to collect DRS, IFS and Raman spectra, respectively. This can use a distal filter and lens system as described previously herein with the distal filters configured for each collection frequency range of the different modes.

FIG. 15C is a schematic diagram of the tissue scanner in accordance with preferred embodiments of the invention. In these embodiments, the housing 1500 contains not only X-Y translation stage 1501, a Dual Deuterium/Halogen source (DRS/IFS) 1502, and a 830 nm Diode Laser (Raman) 1503 but also the spectral analyzers placed externally to the housing in FIG. 15A. Particularly, visible spectrometer 1504, UV miniature spectrometer 1505, and Raman spectrometer 1506, are inside the system housing of the scanner in the embodiment of FIG. 15C. Placement of the spectrometers within the housing can facilitate the system's portable character. As discussed above, the portable device can measure 2x1x1 ft, weighs 30 lbs or less and can easily fit in most clinical spaces including patient examination rooms, procedure rooms and operating rooms. The system can be carried by handle 1509 from one operating room to the next. The glass plate 1508 flattens the tissue surface and provides a reasonably uniform probe-tissue imaging distance. A cover 1512 can cover the sample positioned on the glass plate on tissue support surface. The three probe elements 1514 for reflectance, fluorescence and Raman measurements are mounted in a linear array.

In another preferred embodiment, the system can be configured to have a viewing aperture such that the exposed tissue, such as the skin, of a patient can be scanned by the system. In this embodiment, a patient can be positioned on a table or other support system to enable scanning of a selected region of the patients' tissue.

In lieu of the external data processor 408 depicted in FIG. 15A, the embodiments of FIG. 15C contemplate a touchscreen tablet PC 1507. Touchscreen tablet PC 1507 can be in wireless communication with the system via a wireless (or wired) communication interface located within or on the system. Touchscreen tablet PC 1507 can also have all the capabilities described above with respect to external data processor 408. For example, like external data processor 408, touchscreen tablet PC 1507 can reassemble 2D quantitative hyperspectral DRS and IFS (and Raman) intensity maps of the scanned tissue surface by registering DRS and IFS (and optionally a Raman probe) probe positions. The touchscreen tablet PC can obtain IFS spectra by correcting the raw fluorescence spectra for the effects of tissue absorption and scattering using the corresponding DRS spectra, and analyzed with table PC 1507 using a linear combination model based on MCR. Some of the tablet PC's 1507 operations may be performed onboard the system by a local processor.

Each probe can include a fiber bundle with a single central fiber that delivers excitation light to the tissue, surrounded by a ring with a plurality of optical fibers that collect reflected and fluorescent light returning from the sample and transmit it to the spectrograph (all fibers have

200 μm core and $\text{NA} = 0.22$), terminated with a transparent, protective optical shield. A 75W Xenon arc lamp 403 (Oriel Instrument, USA) and power module 404 can be used to generate excitation light for DRS and a 7 mW Q-switched solid state laser 405 at 355 nm (SNV-401-000, Teem Photonics) with driver circuit 406 is used to generate excitation light for IFS. Other excitation wavelengths can be used for other diagnostic applications. An infrared Raman source 412 can be used for Raman spectral measurements. Signals are collected with miniature spectrometers (USB2000+, Ocean Optics). The spectrometers 407A, 407B, 407C have spectral resolution of 2 nm at full width half maximum (FWHM). The collection fibers 411A, 411B, and 411C are coupled to the corresponding reflectance, fluorescence and Raman spectrometers. The wide area imaging capability is achieved by mechanically scanning optical probes with a long traveling range, XY translation stage 401 and step motors 402 (Applied Motion Products, micro stepper motor: 17-075 and driver: 3540i) in an inverted geometry through a standard glass plate 409 (10 x 12 x 1/16 inches) on which the specimen rests. There is no interference from glass fluorescence with the biomolecular fluorophores of interest, such as collagen and NADH.

The glass plate flattens the tissue surface and provides a reasonably uniform probe-tissue imaging distance. This provides for quantitative measurements, by preserving the key optical characteristics of the probe 410 (spot size and NA), and take full advantage of probe-based spectroscopic models. Excitation beam spot size at the surface of a tissue sample sitting on the glass plate is estimated to be < 1 mm. Labview 8.6 (National Instrument, TX) manages the raster scan by commanding the XY stage 401 through PC serial ports and the spectral data acquisitions. Total scanning time for the tissue sample depends on the choice of scan parameters such as excitation power, integrating time, spatial resolution, field of view, etc., which can be adjusted according to tissue type and clinical need. Note that total scanning time includes timing response to start/stop and reverse the stepper motors. The portable device measures 2 x 1 x 1 ft, weighs 30 lbs or less and can easily fit in most clinical spaces including patient examination rooms, procedure rooms and operating rooms.

DRS, fluorescence spectra (350-700nm), and Raman data can be obtained for each spot scanned. After background subtraction and normalization with 20% spectralon white reflectance standards (Labsphere, NH), DRS spectra are analyzed using a mathematical model based on the diffusion approximation of light propagation in tissue. IFS spectra are then obtained, by correcting the raw fluorescence spectra for the effects of tissue absorption and scattering using the corresponding DRS spectra, and analyzed with data processor 408 using a linear combination model based on multivariate curve resolution (MCR), a standard chemometric method. Spectral modeling provides physically meaningful fitting parameters that are

quantitative measures the contributions of specific tissue components. These spectral parameters are the basis of decision algorithms used in the diagnosis of breast and other cancers. DRS modeling yields 3 scattering parameters: A, which is related to the amount of Mie scatterers; B, which is related to the size of the scatterers; and C, which is related to the amount of Rayleigh scatterers; and absorption fitting parameters for hemoglobin (Hb) and β -carotene, two well-characterized absorbers in breast tissue. IFS modeling yields fluorescence fitting parameters related to NADH, a cellular metabolite, and collagen, a fluorophore that is more abundant in the fibrous stroma of breast cancer than in normal breast tissue.

DRS and IFS data cubes, i.e. three dimensional arrays of image XY coordinates and wavelength, are obtained during each scan. The overlap regions are then co-registered, without the need for complex mathematical transformation except simple shifting of the XY register in acquired pixels, which was previously obtained from the relative position of the probe during calibration. By registration of DRS and IFS (and optionally a Raman probe) probe positions, the processing and display system 408 can reassemble 2D quantitative hyperspectral DRS and IFS (and Raman) intensity maps of the scanned tissue surface. The spectra are then modeled and spectral fitting parameters extracted on a pixel-by-pixel basis to create quantitative parameter maps. A tissue diagnosis can also be rendered using a parameter-based decision algorithm to create diagnostic maps. Currently data is processed off line, and can take up to a second per data point for model fitting. Real-time (on-the-fly) data processing can be performed by incorporating machine learning algorithms that have been used for spectroscopic modalities. Using a multichannel light collection system as described herein can provide scans of 10 cm² area sections in less than 5 minutes.

Tissue-simulating liquid phantoms were prepared from various mixtures of intralipid (Invitrogen), hemoglobin (Hb) (Sigma Aldrich) or blood, and furan (Sigma Aldrich) to validate quantitative extraction of tissue absorption and fluorescence properties from the spectral data obtained with the scanner. Self-adhesive O-rings with an internal diameter of 1 cm were arranged on the glass plate to hold droplets of the liquid phantoms in place. Using a microliter pipette, 200 μ L of each liquid phantom was carefully placed into the O-rings, forming droplets ~2.5 mm in depth. Spectralon standards (10% and 20%) were also placed in the field of view, and were used to normalize the spectral data. Spectra obtained from each spot within each phantom were averaged.

Animal tissue measurements were performed to demonstrate high resolution wide-field hyperspectral imaging capability and spectral contrast to distinguish tissue structures with

5 varying optical properties. A clean-cut cross section of an unfixed, frozen-thawed porcine lower leg tissue specimen (~ 10 cm in diameter) was used. Before placing the tissue section on the glass plate for scanning, it was moistened with normal saline. Porcine lower leg tissue was used for this study as it has more anatomic detail requiring high resolution imaging and a wider range of tissue types with more varied optical properties than breast tissue.

Human tissue measurements were performed using tissue samples of patients. A paired set of unfixed, frozen breast tissues, one grossly cancerous and another matched grossly normal breast tissue from the same patient, were obtained for scanning on the tissue scanner. To prevent dehydration, the tissues were moistened with a small amount of normal saline. The tissues were placed side-by-side on the scanner glass plate. After scanning, the tissue surfaces scanned were marked with colored colloidal inks to preserve orientation, fixed in 10% neutral buffered formalin, processed and embedded in paraffin, and hematoxylin and eosin stained tissue sections prepared for microscopic examination by an experienced breast pathologist, for comparison with the spectroscopic imaging results.

15 Measurements can also be performed on excised melanomas in the skin as well as other solid tumor tissues (e.g. lung, liver, kidney). As these methods can sample from tens to hundreds of microns into the tissue, the system provides improved margin assessment in many applications.

20 A number of measurements were performed to verify system performance. A resolution target was used to demonstrate the imaging capability and to verify spatial resolution of the system. A series of tissue-simulating liquid phantoms was used to validate quantitative extraction of tissue properties from the reflectance and fluorescence spectral data. Moreover, biological tissue was used to illustrate the hyperspectral imaging capability of the system and demonstrate spectral contrast to distinguish tissues with different scattering and absorption properties. Finally, normal and cancer tissues from a breast cancer patient were used to demonstrate the usefulness of the tissue scanner as a cancer margin assessment device.

30 In the resolution target measurement, a positive multi-frequency grid distortion target (NT46-250, Edmond Optics, Fig. 16A) was placed face-down on the glass plate and a DRS scan obtained from an area larger than 2.5 cm x 2.5 cm with excitation power 20 mW, integrating time 20 msec, spatial or image pixel resolution 0.25 mm. Total acquisition time for the DRS image was less than 10 min. At 0.5 μ m wavelength, the smallest dot features printed in the

target, 250 microns in diameter, can be resolved as shown in the randomly selected DRS image at 510 nm in Fig. 16B. Similar resolution was also found for IFS scanning. The measurements show that the tissue scanner can image a large field of view with sub-millimeter resolution. Preferred embodiments utilize a scanning area of at least 16 cm^2 in area (4 cm by 4 cm), for example.

Next, a clean-cut cross section of a frozen-thawed porcine lower leg tissue specimen (~10 cm in diameter) was used to demonstrate scanning of large fields of view and spectral contrast to distinguish tissue structures with different optical properties (Fig. 16C). After placement on the glass plate (Fig. 16D), DRS scans were performed with excitation power 250 mW, integrating time 50msec and spatial resolution 0.5 mm. Total acquisition time for the DRS image was 30min. Figs. 16E-16H show DRS spectral intensity maps acquired with the scanner at selected wavelengths. The DRS spectral intensity map at 487 nm was optimal for distinguishing tissue types with widely differing optical properties in this tissue, including bone, skeletal muscle, fat and connective tissues. The biological tissue measurements show that the tissue scanner can image a large field of view with both high spatial resolution and spectral contrast to distinguish tissues with differing optical properties.

Fifteen liquid tissue simulating phantoms shown in Fig. 17A, composed of a 2% intralipid solution and various amounts of Hb powder to simulate breast tissue scattering and absorption, were prepared and used for DRS calibration. DRS scans with the scanner (Fig. 17B) were performed with excitation power 150 mW, integrating time 100 msec and spatial resolution 0.5mm. Hb concentration is highest in the first phantom (#1) and last phantom (#15), and progressively decreases from phantom 1 to phantom 14. A 2D DRS scanned image of the phantoms and spectralon standards at a randomly selected wavelength (490 nm) is shown in Fig. 17C. As expected, the reflectance signal intensity was inversely proportional to Hb concentration (due to Hb absorption). That is, the reflectance signal is the lowest in phantoms #1 and #15, and increases with decreasing Hb concentration from phantom #1 to phantom #14. Fig. 18A shows a DRS spectrum of a phantom with 1.8mg/ml Hb along with its corresponding model fit and residual. Fig. 18B shows Hb concentration computed from the average phantom DRS spectra, which show excellent agreement with the actual phantom Hb concentrations (error $\leq 5\%$). All calculated scattering-related parameters were constant in all samples. Additional measurements with constant Hb concentration and varying intralipid scatterer concentration showed the computed scattering parameters were proportional to intralipid concentration. The results of these liquid phantom measurements confirm that the

tissue scanner can accurately measure a physiologically relevant range of DRS absorption and scattering parameters across a large scanning field of view.

For IFS validation, 4 liquid tissue simulating phantoms were used, consisting of two replicates each of a 1% intralipid solution with two different concentrations of human blood and furan (0.3 and 0.8 $\mu\text{g/mL}$), which has strong fluorescence in the 400-450 nm region (Table 6). Note that a pin-prick blood sample was used instead of Hb powder to provide additional biochemical fluorophores and scatterers with which to measure DRS correction of the fluorescence spectra for the effects of scattering and absorption. IFS scans were performed with excitation power of 1.5 mW and integrating time of 10 msec. Hb concentrations in the two sets of replicate phantoms were determined from the DRS data and found to be 3.6 ± 0.2 and 0.325 ± 0.25 mg/mL. Fig. 19A shows that the DRS spectra of the 4 phantoms vary primarily with the Hb concentration, whereas the IFS spectra shown in Fig. 19B are largely independent of Hb concentration and depend on the concentration of furan. The results of these liquid phantom measurements confirm that the tissue scanner can accurately measure fluorophores in a variable background of absorption and scattering across a large scanning field of view.

	Furan ($\mu\text{g/mL}$)	Intralipid	Hb (mg/mL)
Phantom 1	0.8	1%	3.80
Phantom 2	0.8	1%	3.40
Phantom 3	0.3	1%	0.35
Phantom 4	0.3	1%	0.30

Table 6. IFS tissue simulating phantom mixtures of furan, intralipid and blood.

To demonstrate the capability of the scanner to identify human breast cancer tissue, a paired set of frozen-thawed breast tissues, one grossly cancerous and another matched grossly normal breast tissue from the same patient, were placed side-by-side on the scanner glass plate as close together as possible to minimize the gap between the tissues (Figs. 20A-20B). DRS and IFS scans were performed by simultaneously scanning the 3 cm x 3 cm (at least 9 cm^2 in area) region of interest, using separate DRS and IFS probes with a probe separation of 0.75 cm and spatial resolution of 0.25 mm per pixel. Excitation power and integrating time were 400 mW and 10 msec for DRS, and 1.5 mW and 10 msec for IFS scanning. Total image acquisition time

for 150 x 150 pixels was 18 mins. 2D quantitative DRS and IFS spectral intensity maps were created. The DRS and IFS spectra were then modeled and fitting parameters extracted to form 2D quantitative parameter maps for comparison with pathology.

Results of pathology examination (Fig. 20C) confirmed that the grossly cancerous tissue consists largely of breast cancer (invasive ductal carcinoma). Interestingly, the grossly normal breast tissue, although seen microscopically to consist largely of normal breast tissue, contained multiple foci of breast cancer (ductal carcinoma in situ) <1 to 3 mm in diameter. DRS and IFS spectra obtained from scanner image pixels occupied by breast cancer and normal breast tissue (Figs. 20D-20E) are similar to those we obtained using similar optical fiber probes in a non-imaging multimodal spectroscopy system. However, although individual 2D DRS and IFS spectral intensity maps demonstrate spectral contrast and have the requisite chemical information embedded in them, they do not by themselves reliably distinguish normal breast tissue from breast cancer (Figs. 21A-21B).

To explicitly distinguish between cancerous and normal breast tissue, employed the physico-chemical fitting parameters extracted from the DRS and IFS spectra. A total of seven parameters were extracted from the spectra and used to characterize the tissue: four DRS parameters (A, B, C, Hb and β -carotene) and two IFS parameters (collagen and NADH). Fig. 22 is a graphical representation of the mean and standard deviation of these spectral parameters for normal and cancer breast tissues. The results show that the normal tissue has clearly different mean values of scattering, absorption and fluorescence parameters than the cancer tissue, and statistical t-test analysis shows that these differences are significant for all parameters at the 99% confidential level (Table 7). Specifically, higher values of A, C, β -carotene and NADH parameters are found in the normal breast tissue, whereas higher values of B, Hb and collagen parameters are found in the breast cancer tissue. The higher values of the C parameter are consistent with the increased size of tumor cell nuclei, and as a result nucleus-to-cytoplasmic ratio, characteristic of breast cancer (both invasive ductal carcinoma and ductal carcinoma in situ). The higher values of the Hb and collagen parameters are also consistent with the presence of angiogenesis and stromal fibrosis, respectively which are typically seen in breast cancer.

Table 7. T-test of the null hypothesis that means of normal and cancer DRS/IFS parameters are not equal

As with the DRS and IFS spectral intensity maps, the individual DRS and IFS parameter maps do not by themselves reliably distinguish normal breast tissue from breast cancer (Fig. 7C-7H). However, normal breast tissue can be reliably distinguished from breast cancer in the tissue scanner images by employing a decision algorithm based on a combination of DRS and IFS parameters. In this method, the IFS collagen and DRS β -carotene parameters are used to distinguish normal breast tissue from all breast lesions including fibrocystic change, fibroadenoma and cancer. Since this measurement includes only normal and cancerous breast tissue, this same process provides adequate diagnostic

Fitting Parameter	h (99%)	p Value	Confidence Interval	tstat Value	Degree of Freedom	Pooled Sample SD
A	1	0	[0.1872 - 0.2250]	28.1151	11496	0.3882
B	1	0	[-0.5644 - (-0.5201)]	-63.0668	8933	0.4062
C	1	0	[0.1453 - 0.1686]	44.3469	9623	0.1662
Hb	1	0	[-1.1695 - (-0.7850)]	-19.7833	11368	2.597
β-carotene	1	0	[0.0004 - 0.0017]	3.9941	10795	0.0129
Collagen	1	0	[-23.4554 - (-19.79)]	-30.3962	9845	34.4264
NADH	1	0.0079	[0.0281 - 1.8752]	2.6558	3462	8.695

discrimination, if it is transferable (i.e. is robust). The parameter scatter plot and diagnostic map in Figs. 23A and 23B show that the DRS-IFS algorithm is transferable and is sufficient to distinguish the tissue types. Representative data points were randomly selected from the two tissue regions for the parameter scatter plot shown in Fig. 23A. The diagnostic map in Fig. 23B identified not only the large focus of invasive ductal carcinoma in the grossly cancerous breast tissue (on the left), but also small (<1 to 3 mm) foci of ductal carcinoma in situ in the grossly normal breast tissue. This indicates that the tissue scanner has sufficient spatial resolution and spectral contrast to detect small foci of cancer in surgical margins.

Thus, a portable, quantitative, multimodal, optical fiber probe-based spectroscopic tissue scanner provides a wide field of view, high resolution and quantitative hyperspectral imaging capability. This exemplifies using a resolution target, tissue-simulating phantoms, animal and

human breast cancer tissue. These measurements show that the tissue scanner has sufficient spatial resolution and spectral contrast to detect small foci of cancer in surgical margins.

The tissue scanner enables quantitative spectroscopic measurements without direct contact with the tissue (thereby enhancing its reproducibility), while maintaining a fixed tissue-probe geometry (which reduces extraneous variance from being incorporated in the measurement). It is a simpler design than those of reflectance and fluorescence imaging systems that utilize a complex array of multi-channel optical fiber probes or an operating microscope for signal collection or utilize a high resolution confocal microscope or micro-endoscope to achieve high spatial resolution. Finally, the tissue scanner combines DRS, IFS and Raman with spectral modeling to provide a more complete, quantitative assessment of the metabolic, biochemical and morphological state of tissue and, as a result, a more robust tissue diagnosis than can be achieved with either modality alone or by using purely statistical algorithms for disease diagnosis.

The system provides coupling of multiple collection fibers to the spectrograph system to amplify the acquired signal and improve imaging speed. Further, chemometrics and machine learning algorithms can be used to provide real time processing of the spectral data. While tissue scanner provide powerful hyperspectral capabilities with spectral resolution of ~2 nm, such resolution may not be necessary for all cancer diagnostics applications.

Non invasive optical techniques, such as diffuse reflectance spectroscopy (DRS), have been used for quantitative tissue characterization and disease diagnosis as described herein. Specifically, DRS provides an assessment of scattering of a sample (which is primarily a function of density and scattering cross sections of internal structures) as well as absorber composition (hemoglobin and beta-carotene).

One of the major challenges of such diagnosis is the accurate and robust analysis of the spectral data, namely the extraction of the absorber concentration and scatterer information, over a variety of sample optical properties and excitation-collection (optical probe) geometries. To this end, diffusion-approximation based solutions of the radiative transport equations and their variants have been employed due to their analytical approach and simplicity of application. However, these methods are not well suited for application in tissues where absorption is high in comparison to scattering (e.g. in cancer angiogenesis) and where the source detector-separation is small. To overcome these difficulties, certain computational techniques such as inverse Monte Carlo models and higher-order analytic approximations have been applied to analyze data.

A look-up table (LUT) approach based on measurements from physical tissue models for optical property determination even at high absorption levels. The primary advantage of the LUT approach is that it does not require modeling of the probe-sample geometry (which is a major challenge for the aforementioned analytical and computational approaches). It also does not necessitate the alteration of the optical probes or measurement at multiple source-detector separation distances. However, the LUT approach involves iterative fitting of the spectra using a non-linear optimization routine, which is computationally expensive (the typical fit time is of the order of a few seconds). This is particularly problematic when the process is deployed in a spectral imaging platform, where data may need to be routinely acquired and processed from a few thousand points. However, the speed of the prediction process is critical to the success of imaging platform applications including the investigation of full tumor margins in intra-operative cancer assessment.

Preferred embodiments of the present invention utilize optical property determination approach based on a non-linear multivariate calibration (MVC) framework. Specifically, a least-squares support vector machine (LS-SVM) can be used to provide rapid and accurate diagnostic information from diffuse reflectance spectra. Previously, empirical models using multivariate calibration (MVC) schemes, including partial least squares (PLS) and neural networks have been employed for analysis of reflectance spectra. However, PLS does not provide accurate predictions as it is unable to account for the intrinsic nonlinear relationships in light transport in tissue. Furthermore, the present LS-SVM system, which provides a class of kernel machines for non-linear regression, results in a unique and reproducible global solution in contrast to neural networks. Using reflectance spectra collected from physical tissue models over a wide range of optical properties, the prediction accuracy and computation time of LS-SVM is compared with that of PLS and the LUT-approach. Here, PLS is chosen for comparison because of its similarity in approach (MVC) with the present method and fast computation time, whereas LUT is selected for its superior prediction performance. These embodiments have the dual advantage of speed and accuracy of the LS-SVM approach makes it suitable for applications in tissue imaging platforms.

Similar to conventional linear regression modeling, LS-SVM estimation between regressors (x) and the dependent variable (y) can be expressed as:

$$y = w^T x + b \quad (1)$$

in which w is the regression coefficient and b represents the bias. Analogous to the ridge regression solution of other problems, LS-SVM constructs a trade-off in the objective function (Q_{LS-SVM}) between the regression error (e) and the minimization of the regression coefficients for N training samples (to enhance model robustness):

$$Q_{LS-SVM} = \frac{1}{2} \|w\|^2 + \gamma \sum_{i=1}^N e_i^2 \quad \text{where } e_i = y_i - w^T x_i - b \quad (2)$$

where the relative weight of the two penalties is mediated by the regularization parameter, γ , and $\|w\|$ represents the 2-norm of the vector of regression coefficients. The critical step in LS-SVM is the subsequent formulation of a Lagrangian function by incorporating the regression error using Lagrangian multipliers (α_i). Solving the resultant Lagrangian problem, it can be shown that the regression problem reduces to the following:

$$y = \sum_{i=1}^N \alpha_i \langle x_i, x \rangle + b \quad \text{where } \alpha_i = \left(\langle x_i, x_i \rangle + (2\gamma)^{-1} \right)^{-1} (y_i - b) \quad (3)$$

Here, the notation $\langle \dots \rangle$ denotes the inner product of the vectors. In essence, the Lagrangian multipliers mediate the relative importance of the individual training samples to the overall model and subsequent prediction. This linear formulation can be readily extended to solve non-linear problems if the inner product is replaced with appropriate kernel functions (K) that satisfy Mercer's conditions. The problem is then modified to:

$$y = \sum_{i=1}^N \alpha_i K \langle x_i, x \rangle + b \quad (4)$$

A polynomial function or a radial basis function can be used for LS-SVM based regression. Clearly, the application of such a function allows higher-dimensional modeling, where the dependent variable estimation of the prediction sample is dependent on the distance of its corresponding regressor from that of the calibration samples in the database.

The kernel functions are parameterized allowing for adjustments with respect to the training data, i.e. the kernel parameter(s) and γ are hyper-parameters specific to the problem dataset. It is worth mentioning that implementation of LS-SVM requires the solution of linear equations as opposed to quadratic optimization for SVM, which makes the former implementation faster. Furthermore, only two parameters need to be optimized for LS-SVM in contrast to three for classical SVM regression, thereby reducing the probability of overfitting the calibration dataset.

For the present embodiment, the diffuse reflectance spectra are input as the regressors (x) to the model whereas the scattering and absorption coefficients are treated as dependent variables (y). Specifically, two models are constructed to predict the scattering and absorption coefficients, respectively, as detailed below.

5 For characterizing the effectiveness of the LS-SVM approach in extracting the optical properties, diffuse reflectance datasets are employed. A reflectance system was used to acquire steady-state spectra (350-700 nm) from a set of 24 physical tissue models (tissue phantoms) with varying absorption (μ_a , 0.5 to 3.79 mm^{-1}) and reduced scattering (μ_s' , 0.44 to 6.74 mm^{-1}) coefficients. A pulsed xenon flash lamp was used as the excitation source and an imaging
10 spectrograph-CCD combination was used for spectral acquisition. A six-around-one fiber-optic probe was used for delivery and collection of light by placing it in close proximity ($\sim 300 \mu\text{m}$) of the samples. The tissue phantoms were fabricated by adding 1 μm polystyrene spheres and India ink to water solutions to simulate typical absorption and scattering properties of biological tissue. Three spectral acquisitions from each sample were averaged for enhanced SNR prior to
15 off-line spectral processing for background correction and normalization using a reflectance standard. The spectral resolution of the system was observed to be approximately 0.78 nm.

The measured dataset consisted of 24 samples in all, was split into calibration and prediction. A leave-one-out protocol was employed to estimate the average prediction error from the 24 samples. For each such splitting, LUT, PLS and LS-SVM models are first generated on
20 the calibration set, by optimizing the model parameters. The optimization of each of these models is performed by inner cross-validation within the calibration dataset. The calibration models are then used prospectively on the prediction data point to determine the absorption (μ_a) and reduced scattering (μ_s') coefficients. In one embodiment it is assumed that the functional form for the reduced scattering coefficient can be: $\mu_s'(\lambda) = \mu_s'(\lambda_0) \cdot (\lambda/\lambda_0)^{-B}$, where λ_0 is a
25 reference wavelength (e.g. 630 nm) and B represents the wavelength dependence of the scattering coefficient. Since the same scatterers were used in the tissue phantoms, the B parameter (which depends on the shape/size and relative refractive index of the scatterers) remains constant for all samples. Additionally, for the dataset, the computation of the absorption coefficient is identical to that of the India ink concentration due to the lack of other significant
30 absorbers. Thus, these computations involved the extraction of the following two values, namely $\mu_s'(\lambda_0)$ and absorber concentration (which can be directly translated to the absorption spectra by multiplying the intrinsic absorption spectrum of India ink). Note that the B parameter can be

readily determined from the computation of the scattering coefficient at a wavelength different from λ_0 using an additional model.

To form the LUT model, the diffuse reflectance values (at all wavelengths) are mapped onto a two-dimensional space constituted by the optical properties at the corresponding wavelengths. Prediction is then performed by executing a non-linear optimization routine which minimizes the error in fitting the sample spectra onto the calibration LUT space (inverse mapping). In contrast to the LUT approach, which creates a single coupled model of the relevant parameters (reflectance values, absorption and scattering coefficients), for the PLS and SVM calculations two separate models for scattering and absorption are constructed. For the PLS regression analysis, the regression vectors for prediction are constructed by employing the number of loading vectors which corresponds to the minimum error in inner cross-validation in the calibration dataset. In these cases, the number of factors is observed to vary between 4 and 7, which ensures that the rank of the calibration model is more than three times smaller than the size of the calibration dataset. The LS-SVM computations are performed using a LS-SVM MATLAB toolbox. Here, a RBF kernel with a typical Gaussian profile ($K(x_i, x_j) = \exp(-\|x_i - x_j\|^2 / 2\sigma^2)$) is used for non-linear regression. As the reflectance spectra can be previously normalized using a reflectance standard, further intensity scaling is not required. Note that the normalization step is important to the reduction of skewed behavior of specific pixel intensity values that reside in greater numeric ranges over those having smaller numeric values. The optimal model parameters γ and σ^2 that provide the smallest error in inner cross-validation are determined by performing a grid search over the range of 1 to 10000 (γ) and 0.01-10 (σ^2), respectively.

In addition to the prediction errors, the average computation time for the prediction step is noted for comparison of LUT, PLS and LS-SVM methods. These computations can be performed on an Intel Celeron(R) CPU 2.00 GHz and 3GB RAM computer, for example.

An objective of the LS-SVM approach to measure optical properties, especially in highly attenuating biological media. The scattering and absorption coefficients and the corresponding fits (using all the three modeling approaches) from a representative tissue phantom are shown in Figs. 24A and 24B. From these figures, one can observe that all the three approaches are valid and can achieve a high level of accuracy. This is also evidenced by the absence of significant features in the accompanying residual plots. However, it is also clear that compared to LUT and LS-SVM based system, PLS provides inferior results, especially for absorption.

To evaluate these results, a leave-one-out validation method, can be used to obtain an average estimate of the prediction errors over the range of measured optical properties. Note that the limited number of samples of tissue phantoms does not allow for the formation of a complete prospective prediction set. Nevertheless, the presence of an outer loop for validation of the calibration models provides a measure of the ability of the model(s) to generalize relationship between the spectra and optical properties beyond the specific data of the calibration set. Figs. 25A and 25B show the box plot results of validation error percentage values obtained for the scattering and absorption coefficients. The percentage error ($(\text{lactual}-\text{predicted})/\text{lactual} \times 100$) is completed instead of the absolute errors due to the large variations in absolute values of these parameters. The mean prediction error percentages for the reduced scattering coefficient are 9.73%, 2.57% and 0.79% for the PLS, LUT and LS-SVM models, respectively. Similarly, the mean prediction error percentages for absorption coefficients are 29.03%, 7.94% and 3.77% for the PLS, LUT and LS-SVM models respectively. Both the approaches substantially improve prediction accuracy as compared to PLS modeling. Furthermore, there is also a corresponding reduction in the standard deviation of the error percentages for both scattering and absorption parameters on application of LS-SVM regression.

These results indicate that PLS is inadequate for modeling of diffuse reflectance spectral data sets, especially in highly attenuating media. This is not surprising given the intrinsic nonlinear relationship between diffuse reflectance and absorption and scattering properties, even under assumptions of semi-infinite media. The specific geometry of the probes, including the source detector separation distance, adds another layer of complexity which necessitates the consideration of nonlinear approaches. Note that curved effects can be modeled by linear multivariate calibration methods such as PLS, but these necessitate the retention of a larger number of factors, with the subsequent risk of inclusion of irrelevant sources of variance in the model. In contrast, LS-SVM based regression is able to address the presence of curved effects, similar to that observed for Raman spectra acquired in turbid media.

Importantly, note the statistically significant improvements in prediction accuracy on application of LS-SVM over the LUT model for both reduced scattering ($p < 10^{-5}$) and absorption ($p = 0.028$) coefficients. This can be attributed to the ability of support vector machines to distinguish between important and irrelevant training samples by assigning corresponding weights (Lagrangian multipliers) to them. This provides the necessary flexibility to deal with outliers, which unduly influence least squares methods (an integral part of the nonlinear fitting routine in the LUT prediction step). The small prediction errors of the LS-SVM model can be

largely attributed to the uncertainty in optical property estimation (based on the gold standard measurement) in the calibration samples and the imperfect construction of the tissue phantoms.

5 A further objective is to examine the computation time taken by the models to predict the tissue optical properties. Fig. 26 gives the computation time in milliseconds for the three modeling approaches. Clearly, the time taken by the LUT approach is greater than that of PLS and LS-SVM models by more than two orders of magnitude. Although appropriate utilization of the memory cache can reduce the computation time of the LUT approach, nevertheless this remains a major challenge for imaging of large sections of biological tissue. Additionally, in contrast to the PLS and LS-SVM methods, there is an inherent tradeoff between the prediction accuracy and the computation time of the prediction step in the LUT approach. Specifically, 10 construction of a larger LUT (i.e. with finer resolution) provides improved prediction accuracy at the cost of increased computation time.

Note that PLS is marginally faster than LS-SVM based regression in predicting tissue optical properties but this advantage is heavily outweighed by its poorer prediction performance. 15 Thus, LS-SVM provides an improved combination of accuracy and computation time for the determination of tissue optical properties. SVM can also be used in the analysis of Raman spectra as described in detail in U.S. Application No. 13/167,445 filed on June 23, 2011 and in International Application PCT/US2011/046750 filed on August 5, 2011, the entire contents of the above applications being incorporated herein by reference. Thus, SVM can be used in a plurality of light detection modes to improve diagnostic accuracy. 20

The LS-SVM system provides a method for rapid and accurate determination of tissue optical properties. Measurements indicate that the LS-SVM regression models are able to maintain a high degree of prediction accuracy over a wide range of tissue properties, which is particularly advantageous for the characterization of highly absorbing tissue, a surrogate marker 25 for tumor growth. This method can be used for the estimation of tissue optical properties without further modification. Given the necessity for faster algorithms for tissue imaging applications, preferred embodiments can be extensively employed in histo-pathological assessment including the detection of tumor margins in breast cancer, cervical pre-cancer diagnosis and other cancers including skin, brain or gastrointestinal tract. This system is adaptable to a wide array of tissue properties and probe geometries. Preferred embodiments can be used for the direct 30 determination of absorber concentration, without *a priori* knowledge of the presence of other absorbers, in applications where the concentration of the analyte of interest (specific absorber) is known (implicit calibration).

While the present invention has been described here in conjunction with a preferred embodiment, a person with ordinary skill in the art, after reading the foregoing specification, can effect changes, substitutions of equivalents and other types of alterations to the system and method that are set forth herein. Each embodiment described above can also have included or
5 incorporated therewith such variations as disclosed in regard to any or all of the other embodiments. Thus, it is intended that protection granted by Letters Patent hereon be limited in breadth only by definitions contained in the appended claims and any equivalents thereof.

CLAIMS

What is claimed is:

1. A system for intraoperative cancer measurement of a region of tissue
5 comprising:
a probe for scanning tissue including a fiberoptic light delivery and light
collection system;
a light source system optically coupled to the probe, the light source system
including a first light source and a second light source;
10 a detector system optically coupled to the probe, the detector system operative
to detect first measured light data from the tissue in response to illumination of the
region of tissue with light from the first light source, and operative to detect second
measured light data from the tissue in response to illumination of the region of tissue
with light from the second light source; and
15 a scanning system operative to actuate relative movement between the probe
and the region of tissue.
2. The system of claim 1, wherein the system weighs 30 lbs or less.
- 20 3. The system of claim 1, wherein the scanning system scans a two dimensional
area of at least 10 cm².
4. The system of claim 1, wherein the scanning system scans a two dimensional
area of up to at least 20 cm by 20 cm.
- 25 5. The system of claim 1, wherein the detector system comprises a first detector
that detects reflected light and a second detector that detects autofluorescence light.
6. The system of claim 1, further comprising a detector that detects Raman
30 shifted light.
7. The system of claim 6, further comprising a data processor that analyzes
spectral data generated by the detector system.

8. The system of claim 7, wherein the data processor performs margin assessment.

9. The system of claim 1, further comprising a data processor configured to implement a non-linear multivariate prediction.

10. The system of claim 9, wherein the multivariate prediction framework is configured to reference diffuse reflectance data, intrinsic fluorescence data, and Raman data.

11. The system of claim 10, wherein the data processor is configured to reference a support vector machine classifier using the diffuse reflectance data, intrinsic fluorescence data, and Raman data.

12. The system of claim 11, wherein the support vector machine comprises a least-squares support vector machine.

13. The system of claim 12, wherein the data processor is configured to use a calibration model to determine the absorption and reduced scattering coefficients of prediction data.

14. The system of claim 7, further comprising a memory storing diffuse reflectance data.

15. The system of claim 7, wherein the data processor is wirelessly coupled to the system.

16. The system of claim 7, the data processor configured to apply a partial least squares classifier.

17. The system of claim 7, wherein the data processor configured to apply a look-up table analysis.

18. The system of claim 7, the data processor configured to apply a neural network classifier.

19. The system of any of claims 7-13, wherein the data processor is configured to perform a decision algorithm, wherein the decision algorithm considers scattering parameters related to an amount of Mie scatterers, a size of the Mie scatterers, and an amount of Rayleigh scatterers.

20. The system of claim 19, wherein the decision algorithm further considers absorption fitting parameters for hemoglobin (Hb) and beta-carotene.

21. The system of claim 20, wherein the decision algorithm further considers fluorescence fitting parameters related to nicotinamide adenine dinucleotide (NADH), a cellular metabolite, and collagen.

22. The system of claim 1, further comprising a display configured to display 2D quantitative reflectance, fluorescence, and Raman intensity maps of the tissue surface.

23. The system of claim 1 further comprising a housing in which the light source system, the scanning system and the detector system are mounted.

24. The system of claim 1 wherein the scanning system comprises a translation stage on which the probe is mounted.

25. The system of claim 1 wherein the probe comprises a probe assembly including a reflectance probe, an autofluorescence probe and a Raman probe.

26. The system of claim 23 wherein the probe is mounted in the housing.

27. The system of claim 25 wherein the probes are mounted in linear array.

28. The system of claim 23 further comprising spectral analyzers for the measured light data mounted in the housing.

29. The system of claim 23 wherein the housing is hand-carried or mountable on a cart.

30. A method for intraoperative cancer measurement comprising:

positioning an tissue sample relative to a fiber optic probe, the fiber optic probe being coupled to a light source system;

actuating relative movement between the tissue sample and the fiber optic probe such that illuminating light from the light source system is scanned across a region of the tissue sample;

detecting light from the region of tissue in response to the illuminating light to generate measured spectral data; and

processing the measured spectral data using a data processing device to determine a spatial distribution of cancerous tissue within the tissue sample.

31. The method of claim 30, further comprising scanning a two dimensional area of at least 10 cm^2 .

32. The method of claim 30 further comprising detecting Raman scattered light from the region of tissue in response to the illuminating light.

33. The method of claim 30 further comprising detecting autofluorescent light from an excised tissue sample in response to the illuminating light.

34. The method of claim 30 further comprising determining a margin of cancerous tissue within the tissue sample.

35. The method of claim 30 further comprising detecting diffuse reflectance light in response to the illuminating light.

36. The method of claim 30 further comprising displaying a spectral image of the tissue.

37. The method of claim 30 wherein the tissue comprises excised breast tissue.

38. The method of claim 30 wherein the tissue comprises skin tissue.

39. The method of claim 30 further comprising positioning the tissue sample on an optically transmissive material and transmitting the illuminating light through the transmissive material in a scan pattern.

40. The method of claim 30 further comprising generating spectral data by detecting Raman scattered light from the region of tissue, detecting autofluorescent light from the region of tissue, and detecting diffuse reflectance light from the region of tissue.

5 41. The method of claim 30 wherein diffuse reflectance light is collected by a first collection fiber and is detected by a first detector, and the fluorescence light is collected by a second collection fiber and is detected by a second detector.

42. The method of claim 41 wherein Raman light is collected by a third collection
10 fiber and detected by a third detector.

43. The method of claim 40 wherein the processing step comprises using a nonlinear representation to process spectral data.

15 44. The method of claim 43 wherein the processing step further comprises using a support vector machine-based classifier to process the spectral data.

45. The method of claim 44 wherein the support vector machine is a least-squares support vector machine.
20

46. The method of claim 30 wherein the processing step further comprises using a partial least squares classifier to process spectral data.

47. The method of claim 30 wherein the processing step further comprises using a
25 look-up table to process spectral data.

48. The method of claim 30 wherein the processing step further comprises using a neural network classifier to process spectral data.

30 49. The method of claim 45 wherein the determining a spatial distribution of cancerous tissue is based upon diffuse reflectance spectroscopy data and intrinsic fluorescence signature data.

50. The method of claim 30 further comprising selecting scan parameters including scan area resolution and scan time.

51. The method of claim 30 further comprising collecting spectral data from a scan area using a single light spot size.

52. The method of claim 30 further comprising executing a decision algorithm based on a combination of diffuse reflectance spectroscopy data and intrinsic fluorescence signature data.

53. The method of claim 52 wherein the decision algorithm considers the amount of Mie scatterers, a size of the Mie scatterers, and an amount of Rayleigh scatterers.

54. The method of claim 53 wherein the decision algorithm further considers absorption fitting parameters for hemoglobin (Hb) and beta-carotene.

55. The method of claim 54 wherein the decision algorithm further considers fluorescence fitting parameters related to nicotinamide adenine dinucleotide (NADH), a cellular metabolite, and collagen.

56. A method for skin cancer measurement comprising:
positioning a skin tissue sample relative to a fiber optic probe, the fiber optic probe being coupled to a light source system;
actuating relative movement between the tissue sample and the fiber optic probe such that illuminating light from the light source system is scanned across a region of the skin tissue sample;
detecting light from the region of skin tissue in response to the illuminating light to generate measured spectral data; and
processing the measured spectral data using a data processing device to determine a spatial distribution of cancerous tissue within the skin tissue sample.

57. The method of claim 56 further comprising scanning a two dimensional area of at least 10 cm².

58. The method of claim 56 further comprising detecting Raman scattered light from the region of tissue in response to the illuminating light.

5 59. The method of claim 56 further comprising detecting autofluorescent light from the region of tissue in response to the illuminating light.

60. The method of claim 56 further comprising determining a margin of cancerous tissue within the tissue sample.

10 61. The method of claim 56 further comprising detecting diffuse reflectance light in response to the illuminating light.

15 62. The method of claim 56 further comprising displaying a spectral image of the tissue.

63. The method of claim 56 wherein the skin tissue comprises excised tissue sample.

20 64. The method of claim 56 further comprising positioning the skin tissue sample on an optically transmissive material and transmitting excitation light through the transmissive material in a scan pattern.

25 65. The method of claim 56 wherein the probe further comprises a first probe element to collect Raman data, a second probe element to collect autofluorescence light and a third probe element to collect reflectance light.

30 66. The method of claim 56 further comprising generating spectral data by detecting Raman scattered light from the region of tissue, detecting autofluorescent light from the region of tissue, and detecting diffuse reflectance light from the region of tissue.

67. The method of claim 66 wherein diffuse reflectance light is collected by a first collection fiber and is detected by a first detector, and the fluorescence light is collected by a second collection fiber and is detected by a second detector.

68. The method of claim 67 wherein Raman light is collected by a third collection fiber and detected by a third detector.

5 69. The method of claim 56 wherein the processing step comprises using a nonlinear representation to process spectral data.

70. The method of claim 69 wherein the processing step comprises using a support vector machine classifier to process spectral data.

10 71. The method of claim 70 wherein the support vector machine is a least-squares support vector machine.

72. The method of claim 56 wherein the processing step further comprises using a partial least squares classifier to process spectral data.

15 73. The method of claim 56 wherein the processing step further comprises using a look-up table to process spectral data.

20 74. The method of claim 56 wherein the processing step further comprises using a neural network classifier to process spectral data.

75. The method of claim 60 wherein the step of determining a spatial distribution of cancerous tissue is based upon diffuse reflectance data and intrinsic fluorescence data.

25 76. The method of claim 56 further comprising selecting scan parameters including scan area, resolution and scan time.

77. The method of claim 56 further comprising collecting spectral data from a scan area using a single light spot.

30 78. The method of claim 56 further comprising executing a decision algorithm based on a combination of diffuse reflectance data and intrinsic fluorescence data.

79. The method of claim 78 wherein the decision algorithm considers the amount of Mie scatterers, a size of the Mie scatterers, and an amount of Rayleigh scatterers.

80. The method of claim 79 wherein the decision algorithm further considers absorption fitting parameters for hemoglobin (Hb) and beta-carotene.

81. The method of claim 80 wherein the decision algorithm further considers fluorescence fitting parameters related to nicotinamide adenine dinucleotide (NADH), a cellular metabolite, and collagen.

82. A system for measuring a region of tissue comprising:
a probe for scanning tissue including a fiber optic light delivery and collection system;
a light source system optically coupled to the probe, the system including at least a first light source for diffuse reflectance and a second light source for fluorescence;
a detector system that is coupled to the fiber optic light collection system to detect reflectance and autofluorescence from a tissue sample; and
a scanning system to provide relative movement between the probe and a tissue sample.

83. The system of claim 82 further comprising a third light source for Raman excitation.

84. The system of claim 82, wherein the probe is mounted on the scanning system to translate the probe relative to the region of tissue of a tissue sample.

85. The system of claim 82 wherein the scanning system provides a scanning region that is at least an area of 4 cm².

86. The system of claim 82 wherein the detector system comprises a first detector that detects reflected light and a second detector that detects fluorescence light.

87. The system of claim 86 further comprising a third detector that detects Raman shifted light.

88. The system of claim 82, wherein the scanning system translates the probe across a scanning region of at least 10 cm² in area with a scanning time of less than 20 minutes.

89. The system of claim 82, further comprising a data processor that analyzes spectral data.

90. The system of claim 89, wherein the data processor performs cancer margin assessment.

91. The system of claim 82, further comprising a data processor configured to implement a non-linear multivariate prediction analysis.

92. The system of claim 91, wherein the multivariate prediction analysis is configured to reference diffuse reflectance data and intrinsic fluorescence data.

93. The system of claim 91, wherein the data processor is configured to apply a support vector machine classifier.

94. The system of claim 93, wherein the support vector machine is a least-squares support vector machine.

95. The system of claim 94, wherein the data processor is configured to use a calibration model to determine the absorption and reduced scattering coefficients of a prediction data point.

96. The system of claim 75, further comprising a memory storing diffuse reflectance data.

97. The system of claim 75, wherein the data processor is wirelessly coupled to the portable system.

98. The system of claim 91, wherein the data processor configured to apply a partial least squares-based classifier.

99. The system of claim 91, wherein the data processor configured to apply a look-up table analysis,

100. The system of claim 91, wherein the data processor configured to apply a neural network classifier.

101. The system of any of claims 82-98, wherein the data processor is further configured to perform a decision algorithm based on a combination of diffuse reflectance spectroscopy data and intrinsic fluorescence signature data.

102. The system of claim 101, wherein the decision algorithm considers the amount of Mie scatterers, a size of the Mie scatterers, and an amount of Rayleigh scatterers.

103. The system of claim 102, wherein the decision algorithm further considers absorption fitting parameters for hemoglobin (Hb) and beta-carotene.

104. The system of claim 103, wherein the decision algorithm further considers fluorescence fitting parameters related to nicotinamide adenide dinucleotide (NADH), a cellular metabolite, and collagen.

105. A method of measuring tissue comprising:
positioning a probe relative to a region of tissue to be measured, the probe having at least one light delivery optical fiber and at least one light collection optical fiber;
illuminating the region of tissue with light from a first light source and a second light source, the light being delivered through the light delivery optical fiber;
collecting light returning from the region of tissue with the at last one light collection optical fiber that collects reflected light and that collects fluorescence light; and
detecting reflected and fluorescence light collected from the tissue at a plurality of positions.

106. The method of claim 105 wherein the tissue comprises excised breast tissue.

107. The method of claim 105 wherein the tissue comprises skin tissue.

108. The method of claim 105 further comprising detecting Raman scattered light.

109. The method of claim 105 further positioning a tissue sample on an optically transmissive material and transmitting excitation light through the transmissive material in a scan pattern.

110. The method of claim 105 further comprising analyzing spectral data to perform cancer margin assessment of the tissue.

111. The method of claim 105 further comprising analyzing the detected light with a data processor.

112. The method of claim 111 wherein the analyzing step further comprises determining whether the tissue is cancerous or normal tissue.

113. The method of claim 111 wherein the analyzing step comprises using a nonlinear representation to process spectral data.

114. The method of claim 111, wherein the analyzing step comprises using a support vector machine classifier to process spectral data.

115. The method of claim 111, further comprising determining whether the region of tissue includes cancerous tissue.

116. The method of claim 115, wherein the determining is based upon diffuse reflectance spectroscopy data and intrinsic fluorescence signature data.

117. The method of claim 105, further comprising actuating relative movement between a fiber optic probe and a tissue sample using a scanning system.

118. The method of claim 117, further comprising selecting scan parameters including scan area resolution and scan time.

119. The method of claim 118, further comprising collecting spectral data from a scan area using a single light spot.

120. The method of claim 105, wherein diffuse reflectance light is detected by a first detector and fluorescence light is detected by a second detector.

121. The method of claim 120, wherein Raman light is collected by a third collection fiber and detected by a third detector.

122. A portable system for measuring a region of tissue comprising:
a probe for scanning tissue including a fiber optic light delivery and collection system,
the probe mounted on the scanning system to translate the probe relative to the region of tissue;

a light source system optically coupled to the probe, the system including at least a first light source for diffuse reflectance, a second light source for fluorescence, and a third light source for Raman excitation;

a detector system that is coupled to the fiber optic light collection system to detect reflectance, autofluorescence, and Raman shifted light from a tissue sample;

a scanning system to provide relative movement between the probe and a tissue sample;

a housing in which the light source system, the detector system and the scanning system are mounted; and

a data processor configured to analyze spectral data.

123. The portable system of Claim 122, wherein the data processor is wirelessly coupled to the portable system.

124. The portable system of Claim 123, wherein the data processor is configured to perform a multivariate calibration.

125. The portable system of Claim 122, further comprising a memory connected to the data processor that stores spectral data storing.

126. The portable system of Claim 122, further comprising a display configured to
5 2D quantitative reflectance, fluorescence, and Raman intensity maps of the tissue.

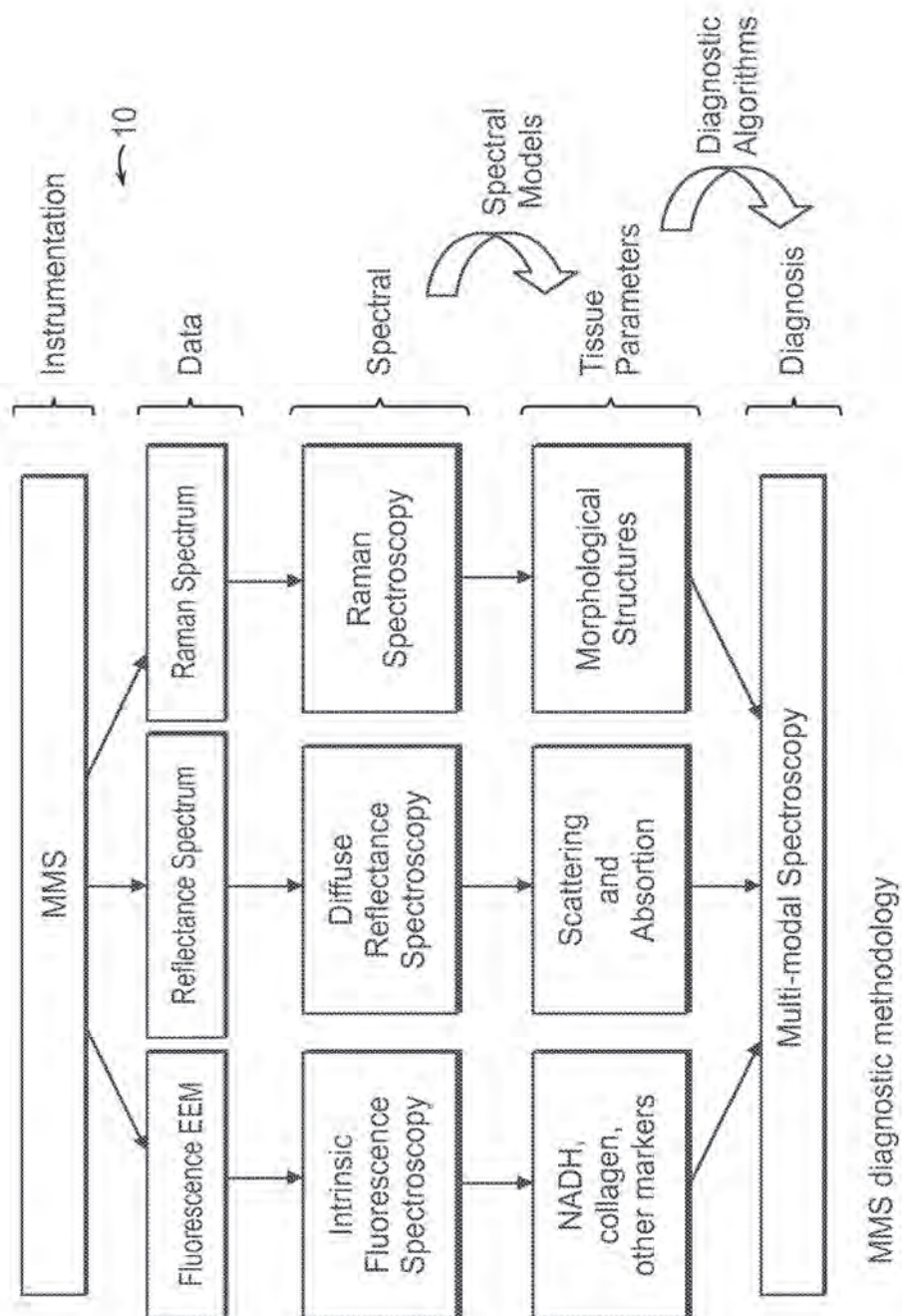


FIG. 1A

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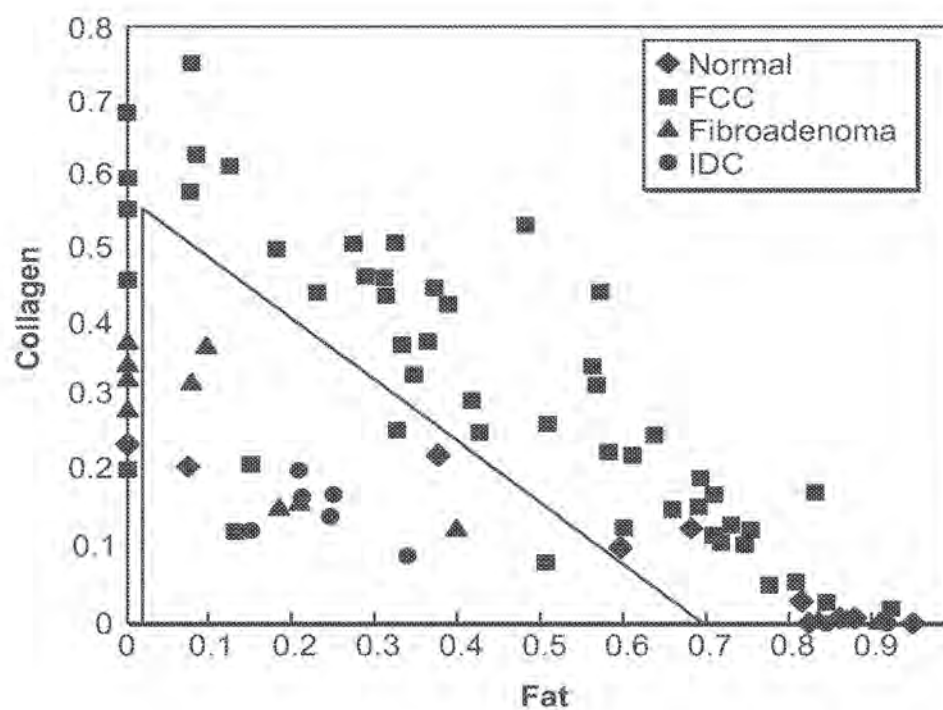


FIG. 1B

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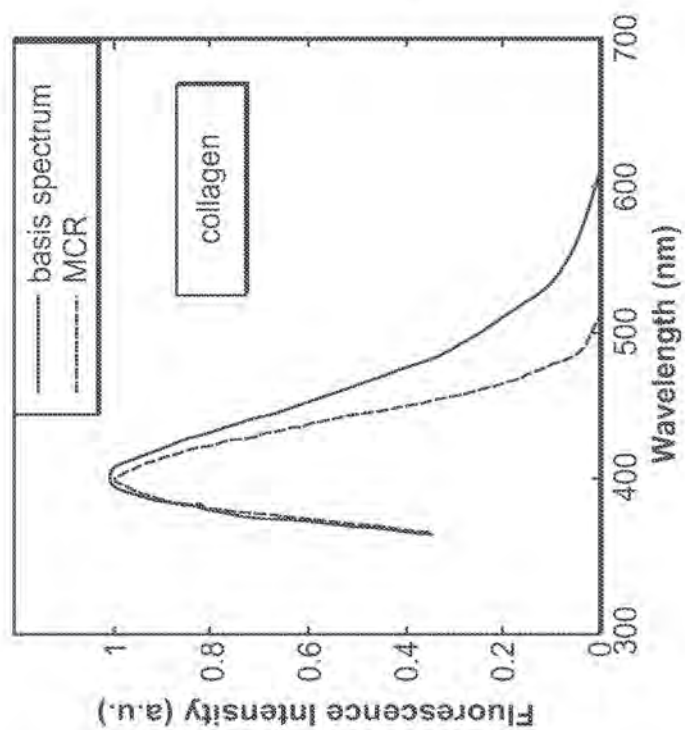


FIG. 2B

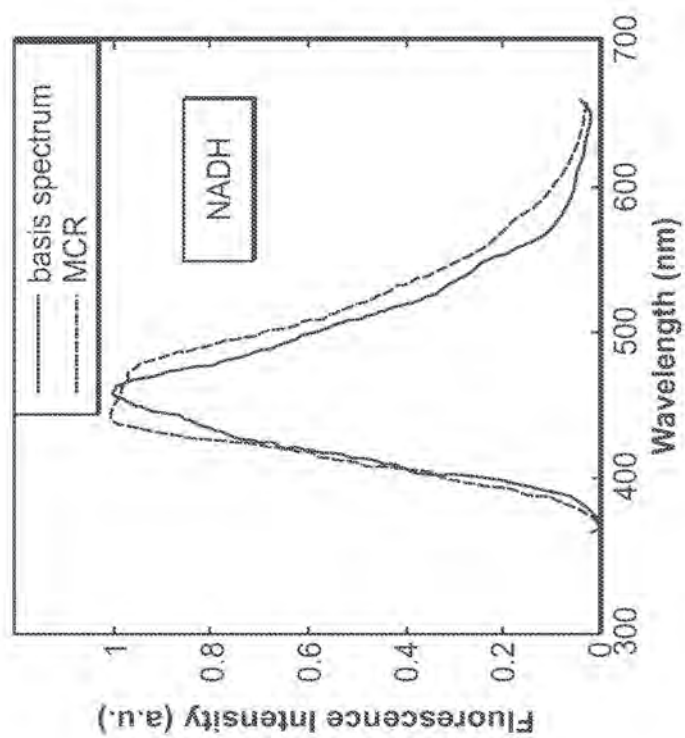


FIG. 2A

SUBSTITUTE SHEET (RULE 26)

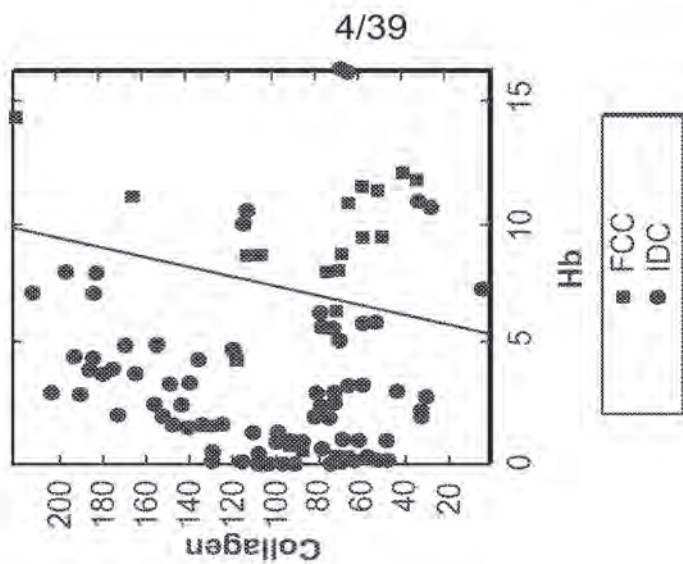


FIG. 3C

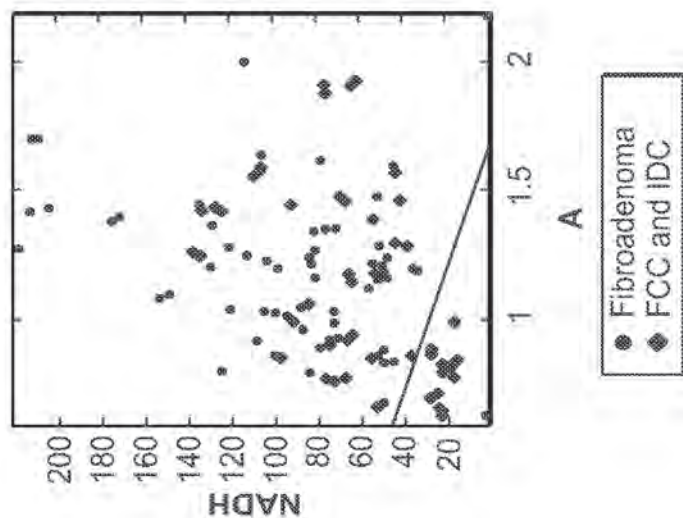


FIG. 3B

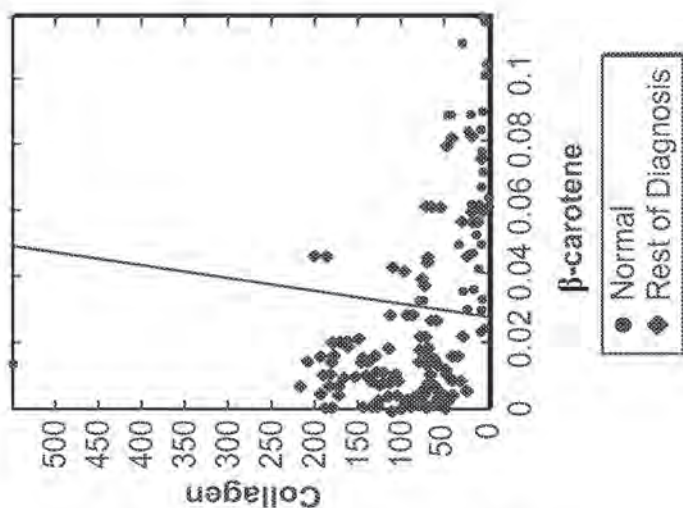


FIG. 3A

SUBSTITUTE SHEET (RULE 26)

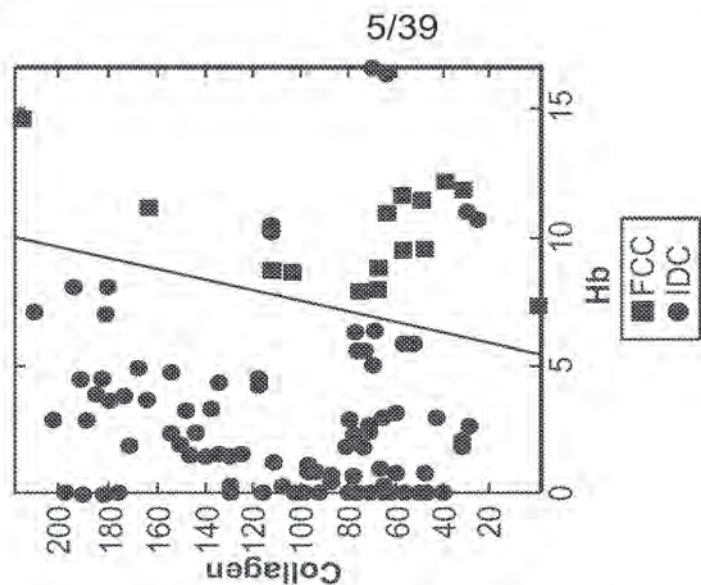


FIG. 4C

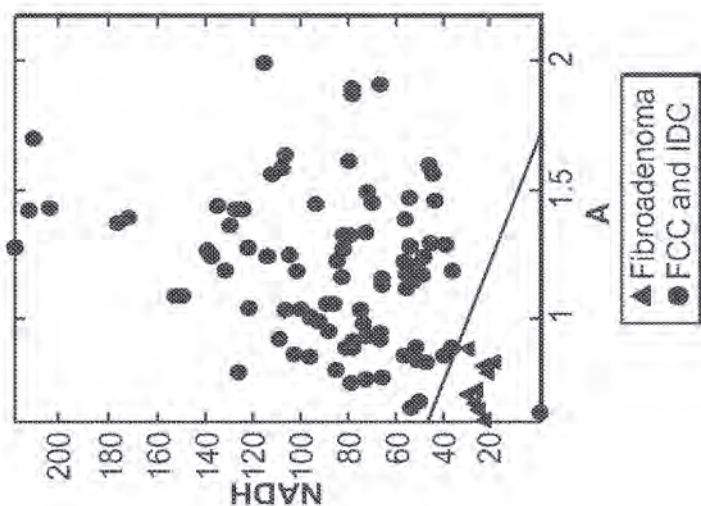


FIG. 4B

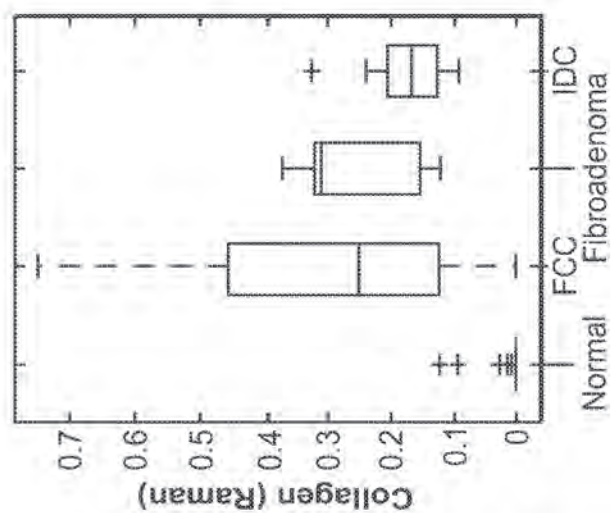


FIG. 4A

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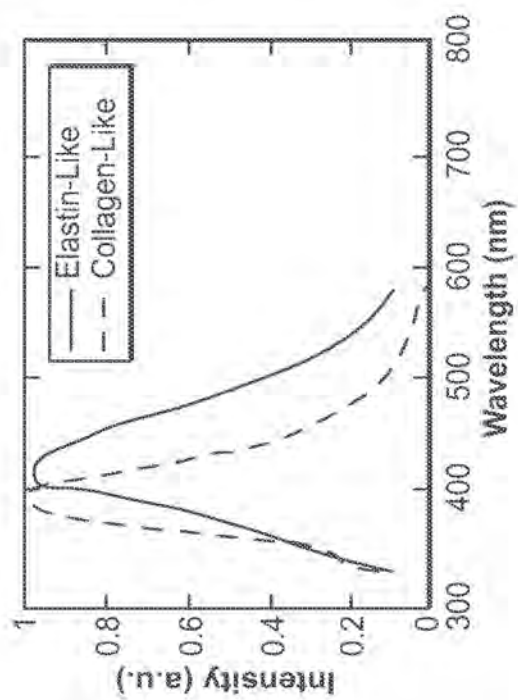


FIG. 5B

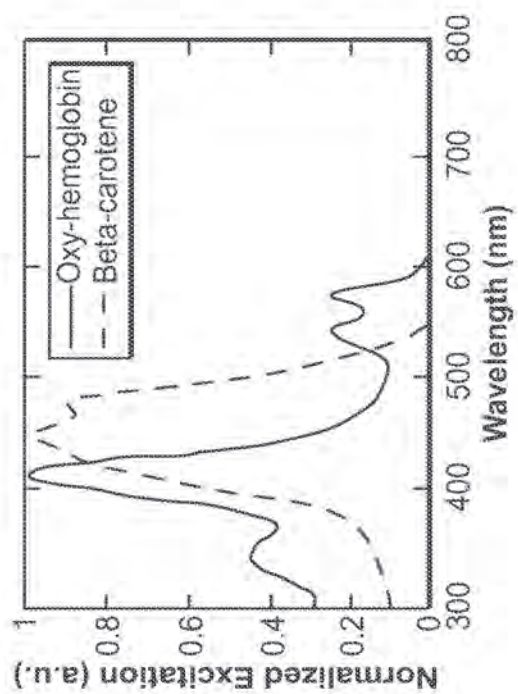


FIG. 5A

SUBSTITUTE SHEET (RULE 26)

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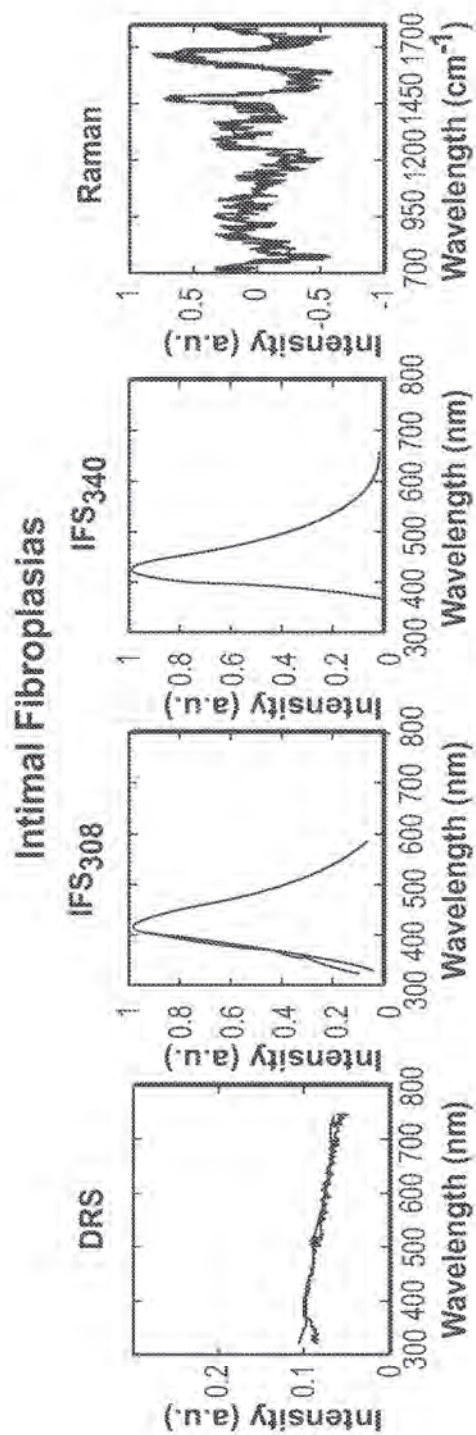


FIG. 6A

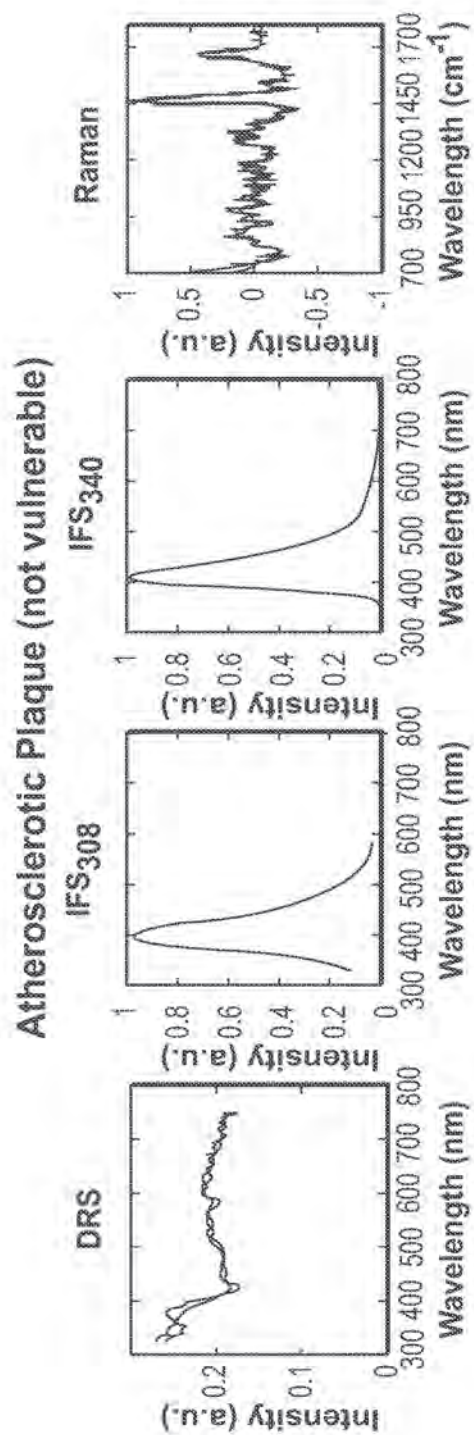


FIG. 6B

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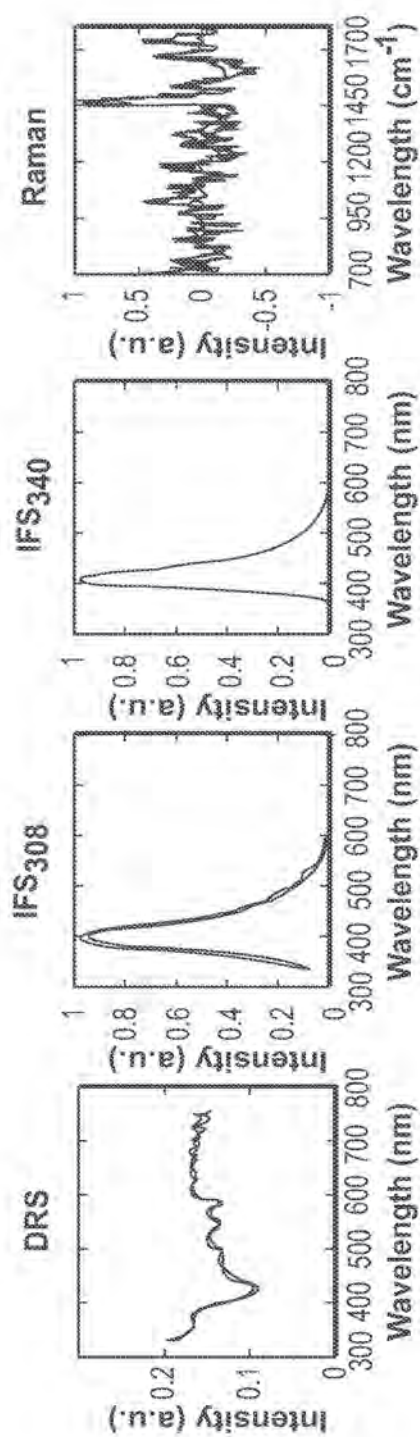


FIG. 6C

SUBSTITUTE SHEET (RULE 26)

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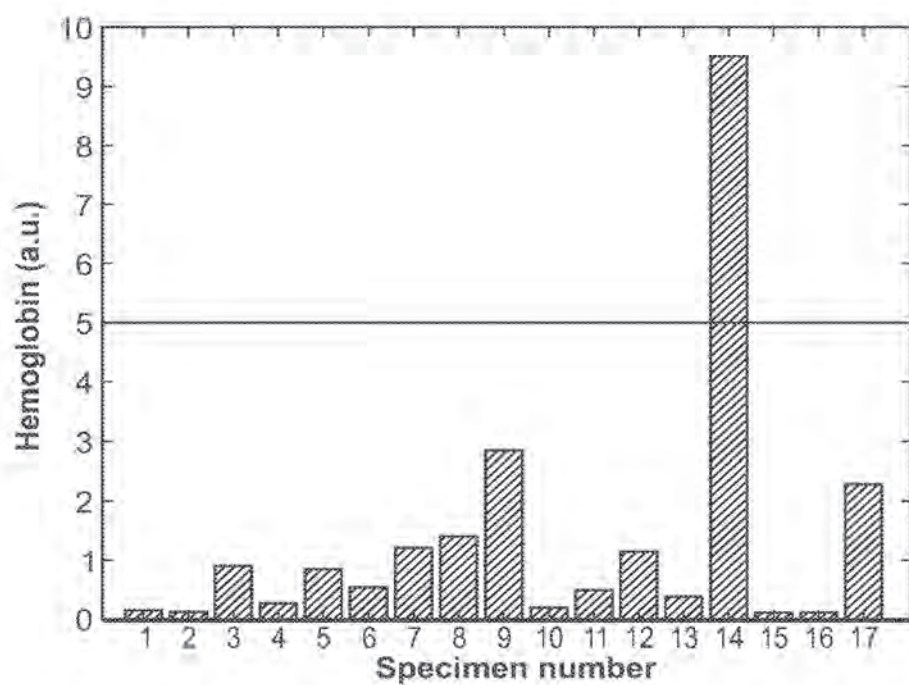


FIG. 7

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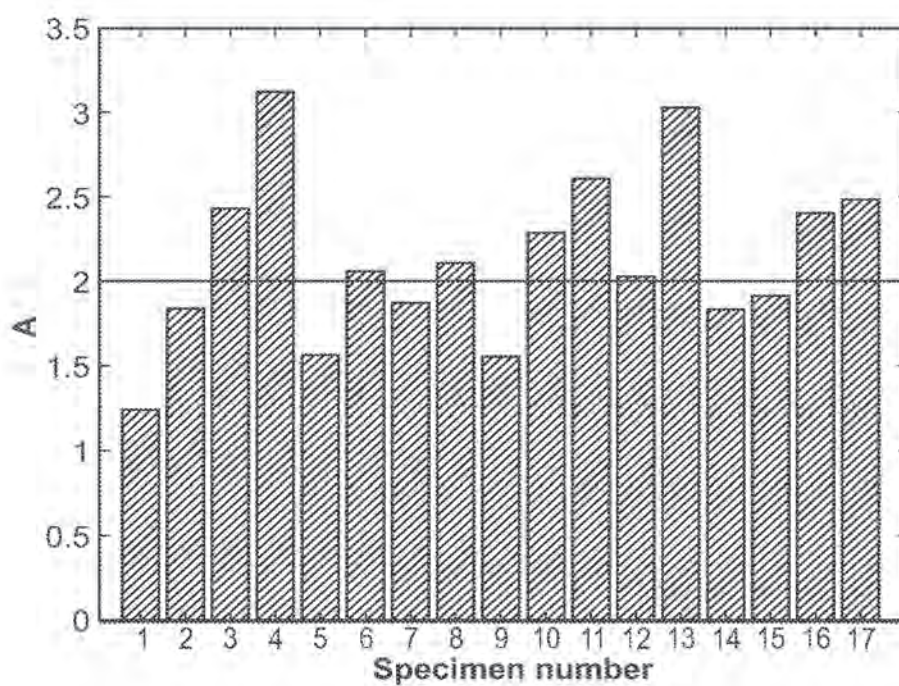


FIG. 8

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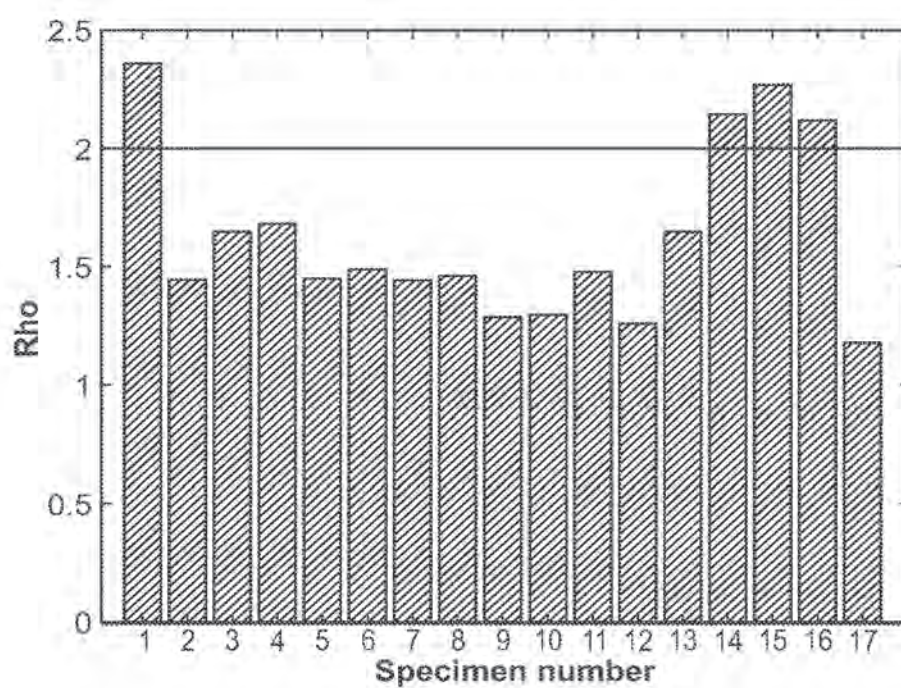


FIG. 9

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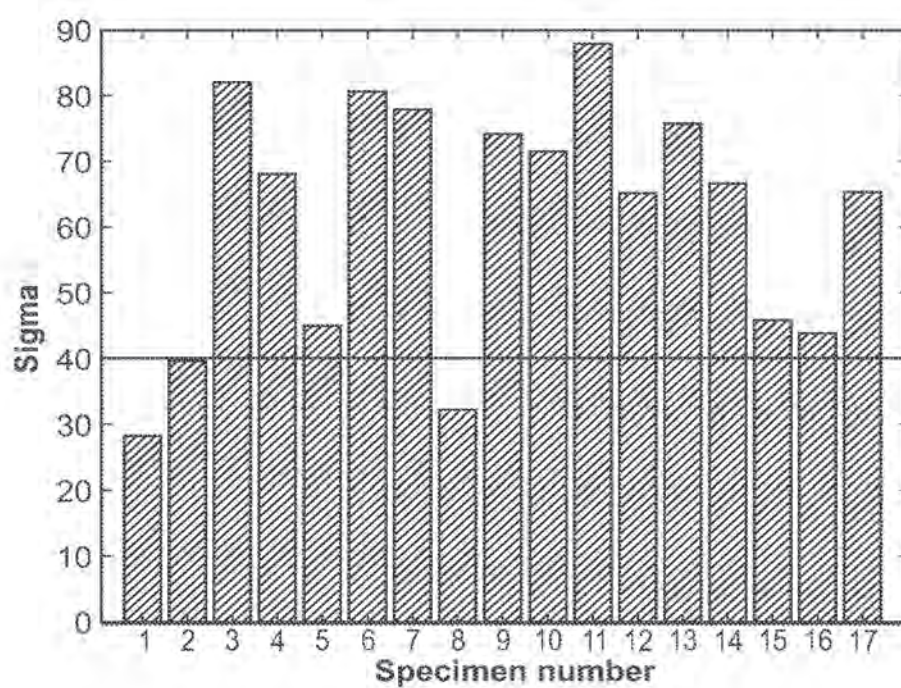


FIG. 10

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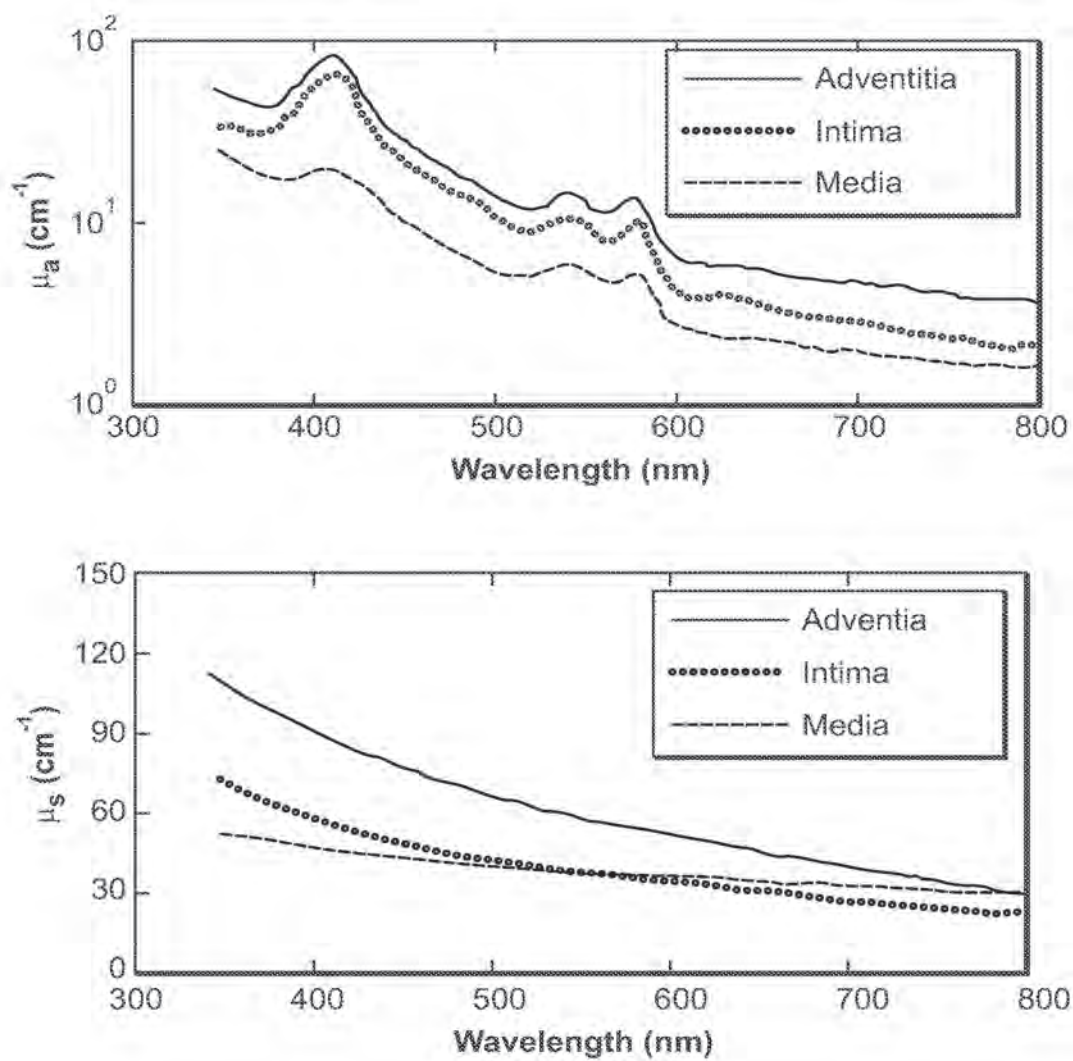


FIG. 11A

SUBSTITUTE SHEET (RULE 26)

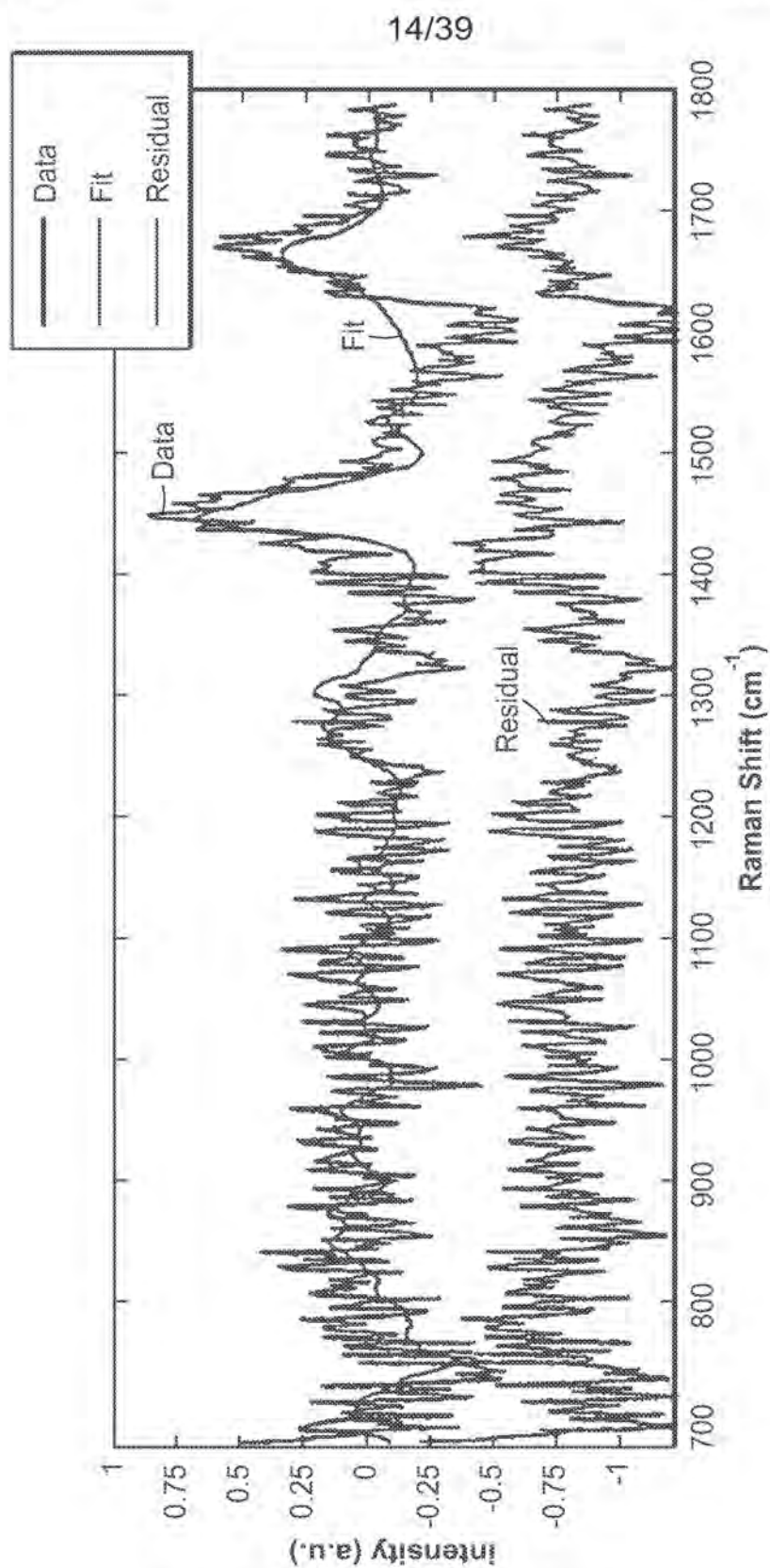


FIG. 11B

SUBSTITUTE SHEET (RULE 26)

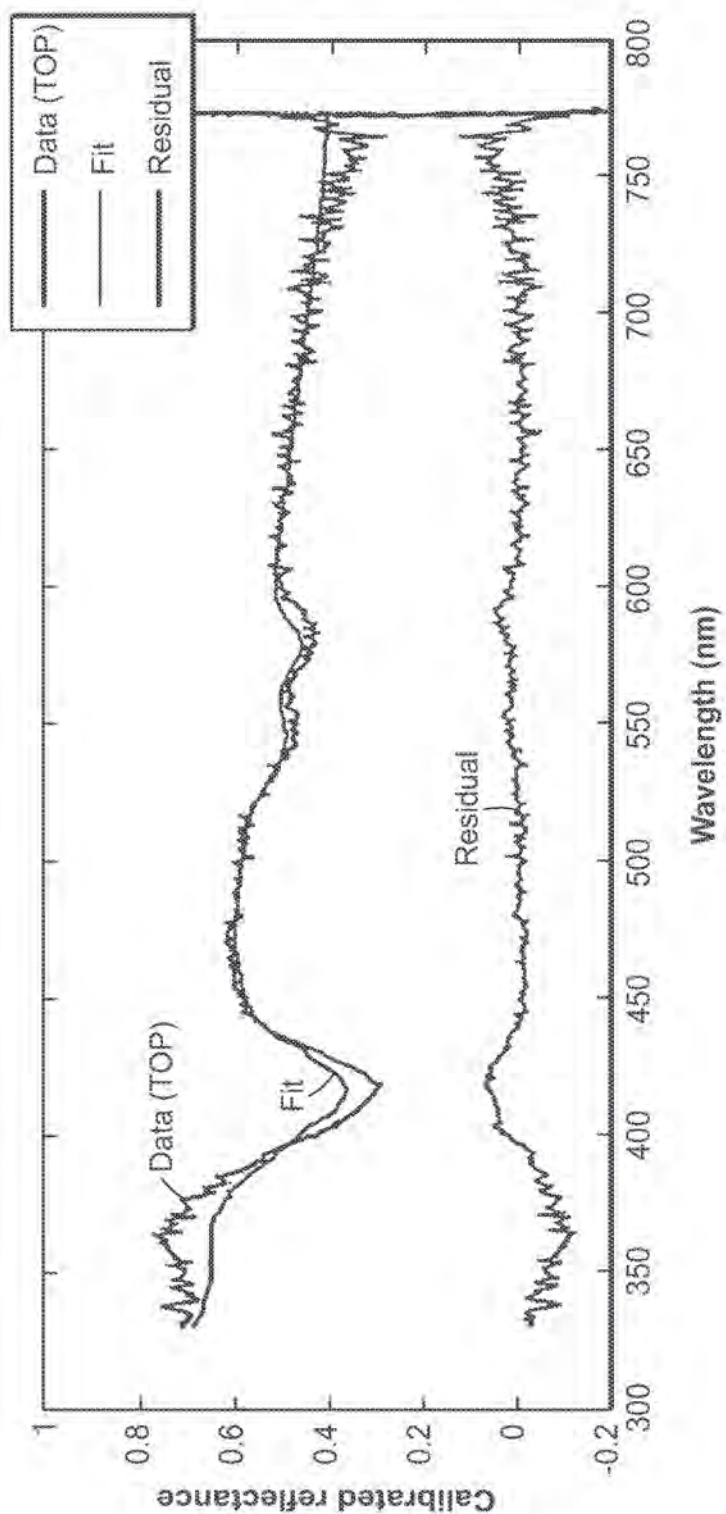


FIG. 11C

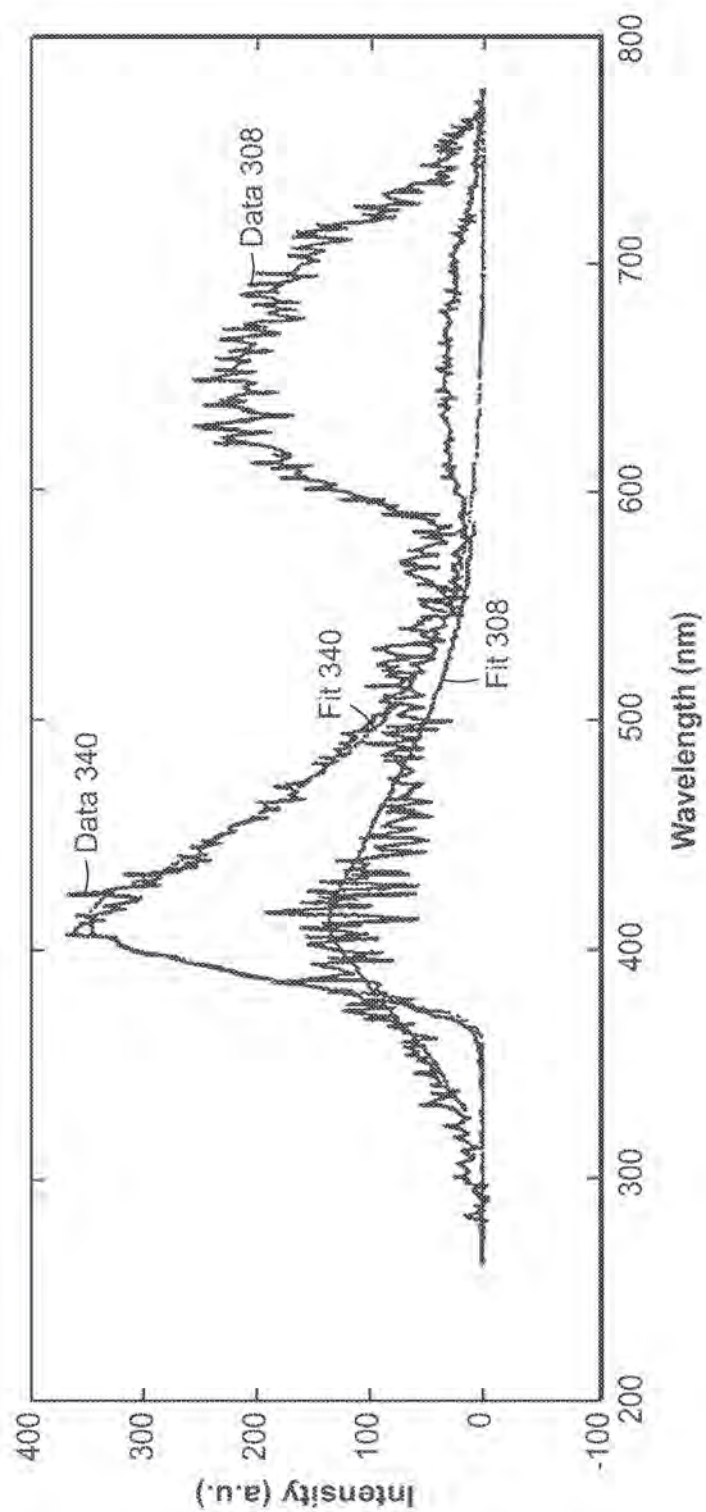


FIG. 11D

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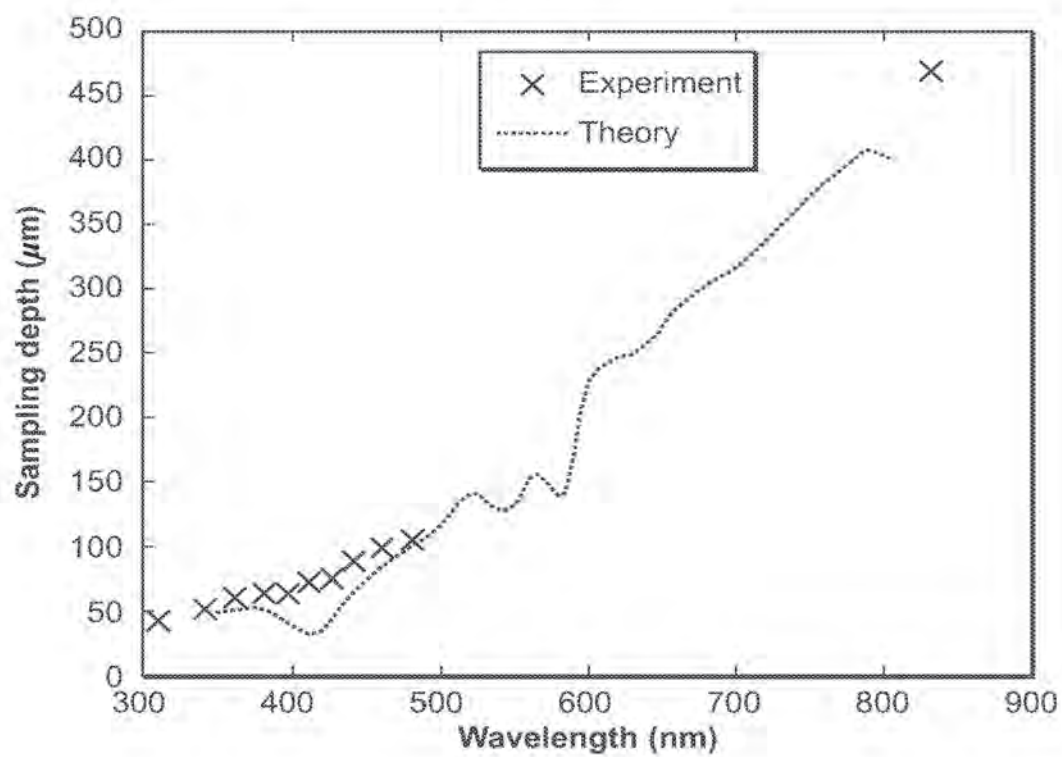


FIG. 12

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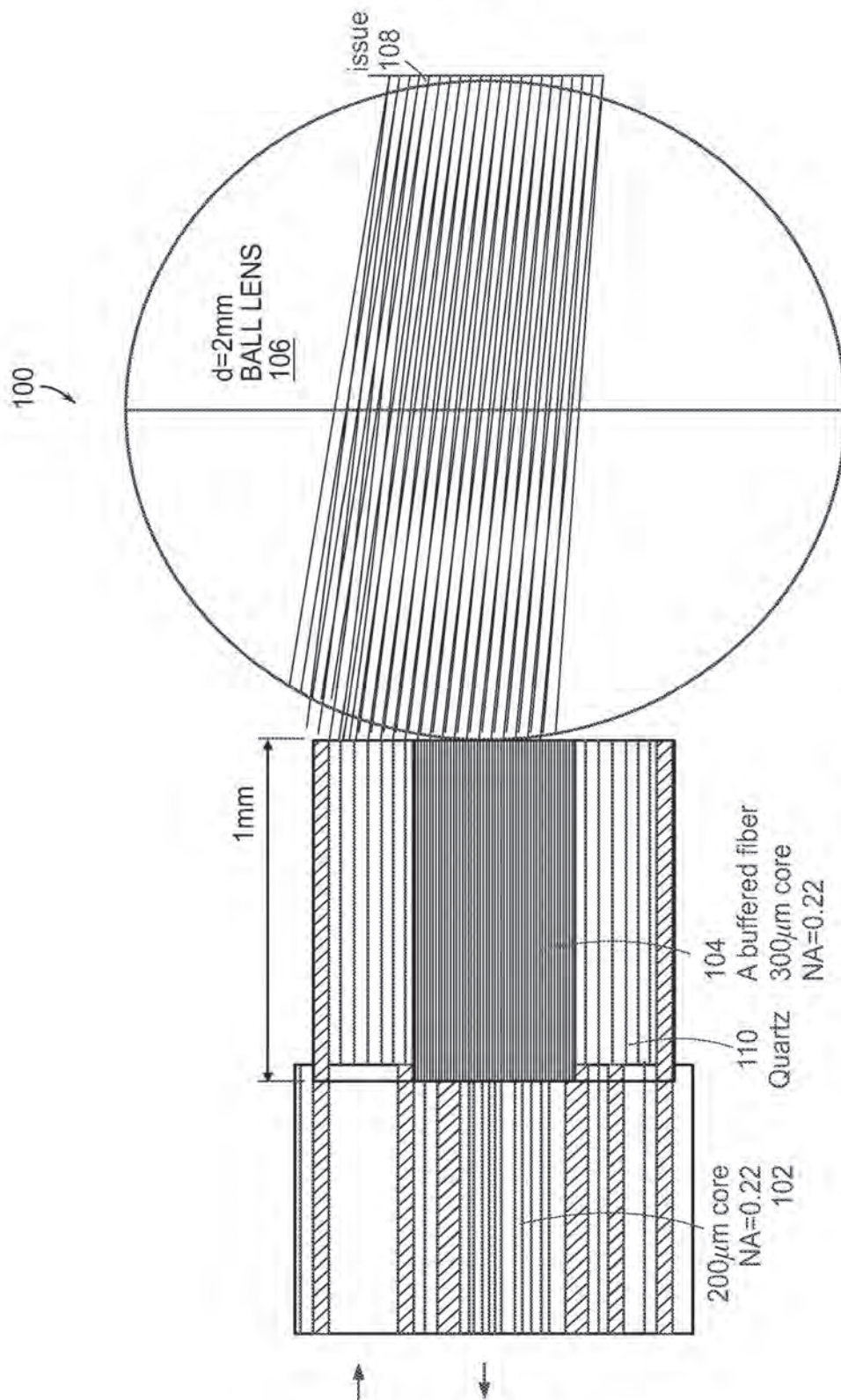


FIG. 13A

SUBSTITUTE SHEET (RULE 26)

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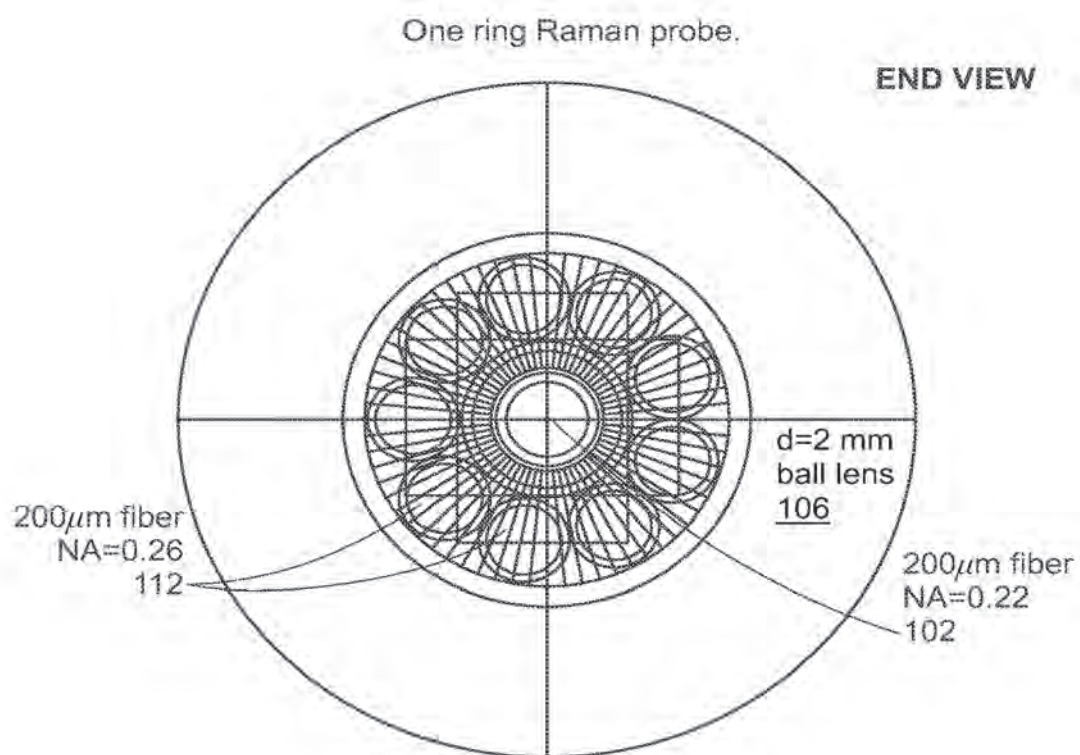


FIG. 13B

SUBSTITUTE SHEET (RULE 26)

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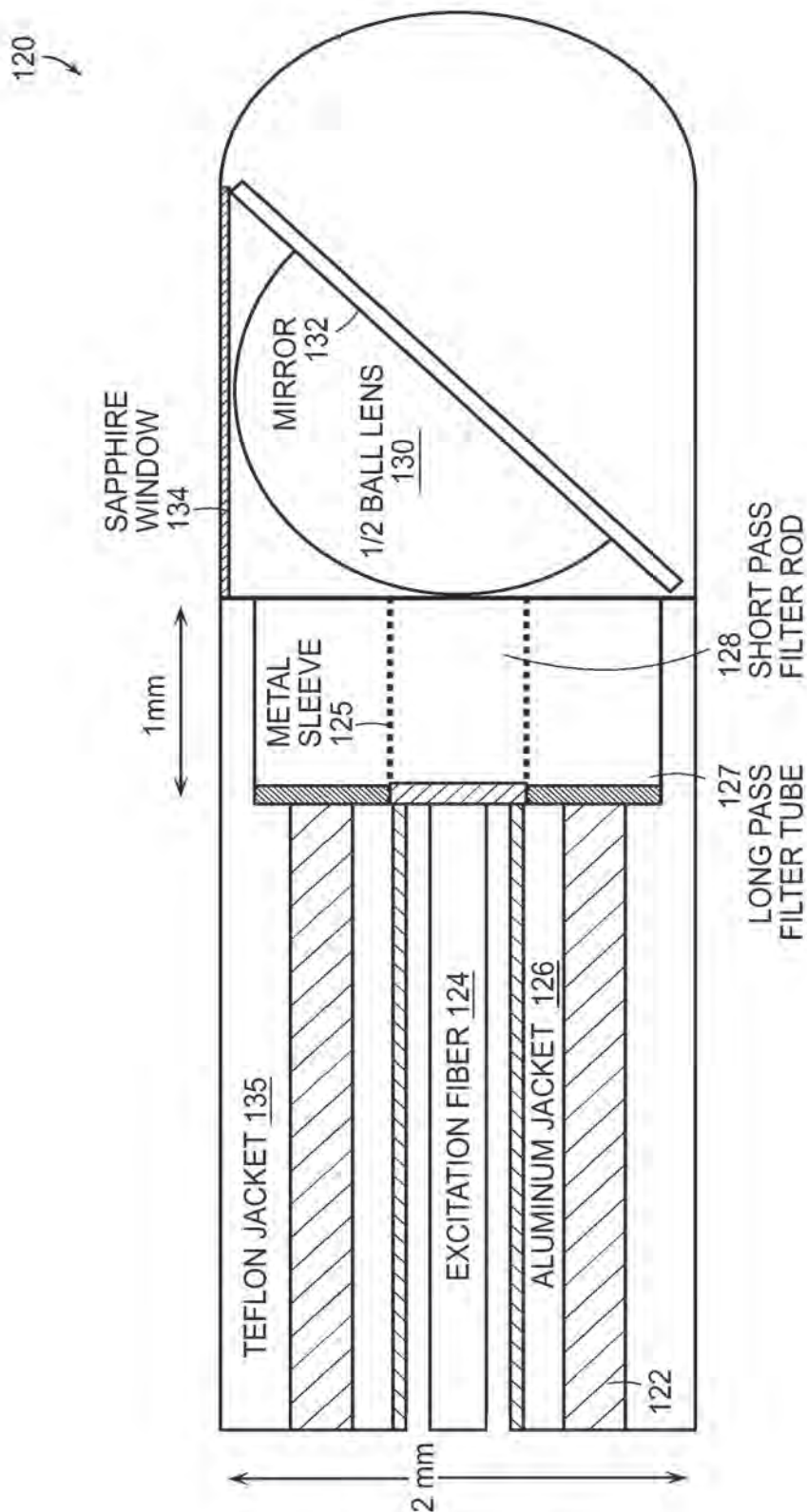


FIG. 13C

SUBSTITUTE SHEET (RULE 26)

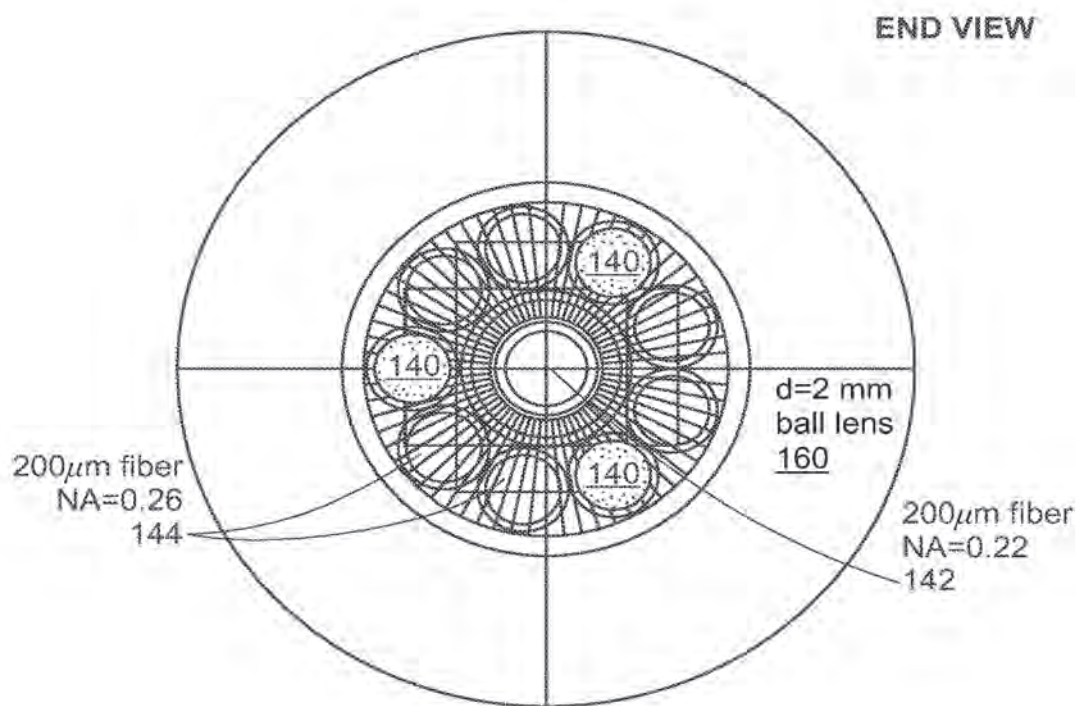


FIG. 13D

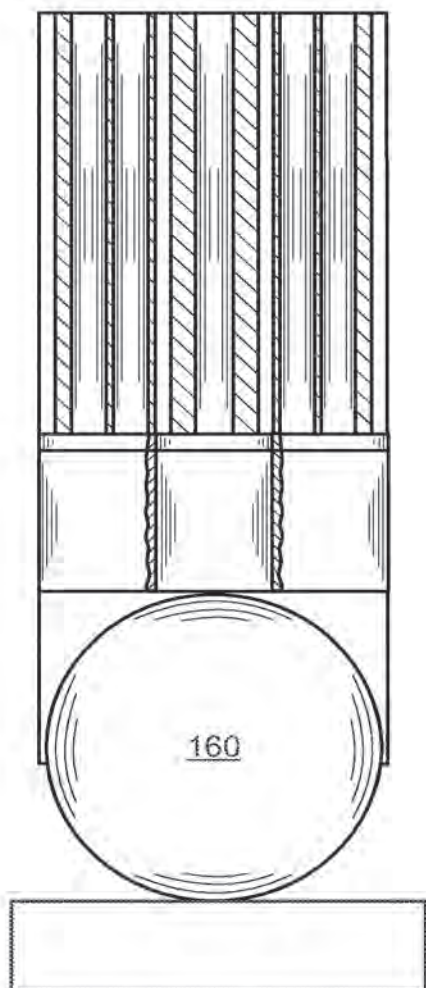


FIG. 13E

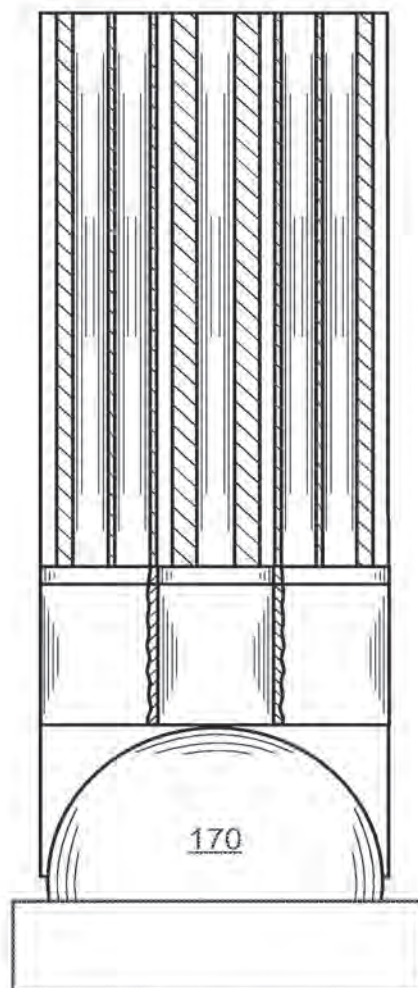


FIG. 13F

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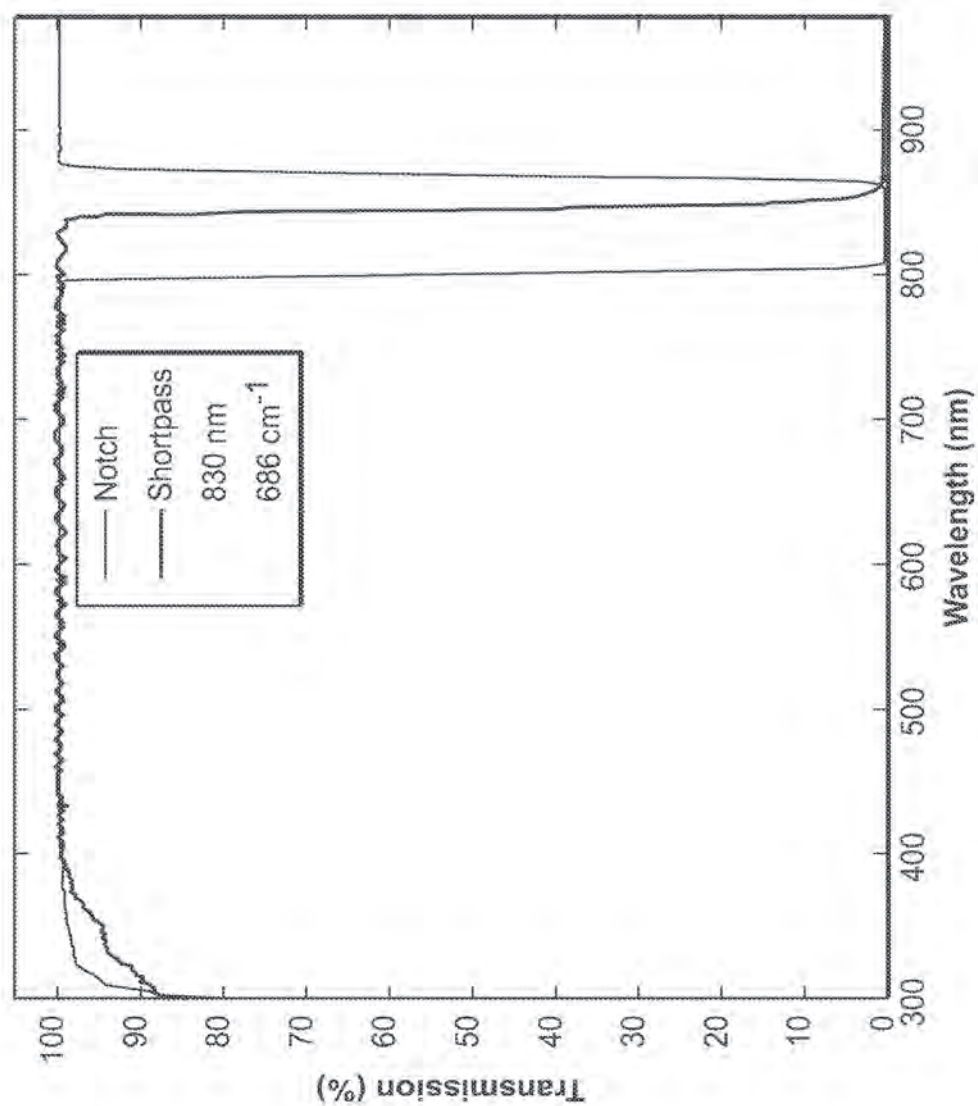


FIG. 13G

SUBSTITUTE SHEET (RULE 26)

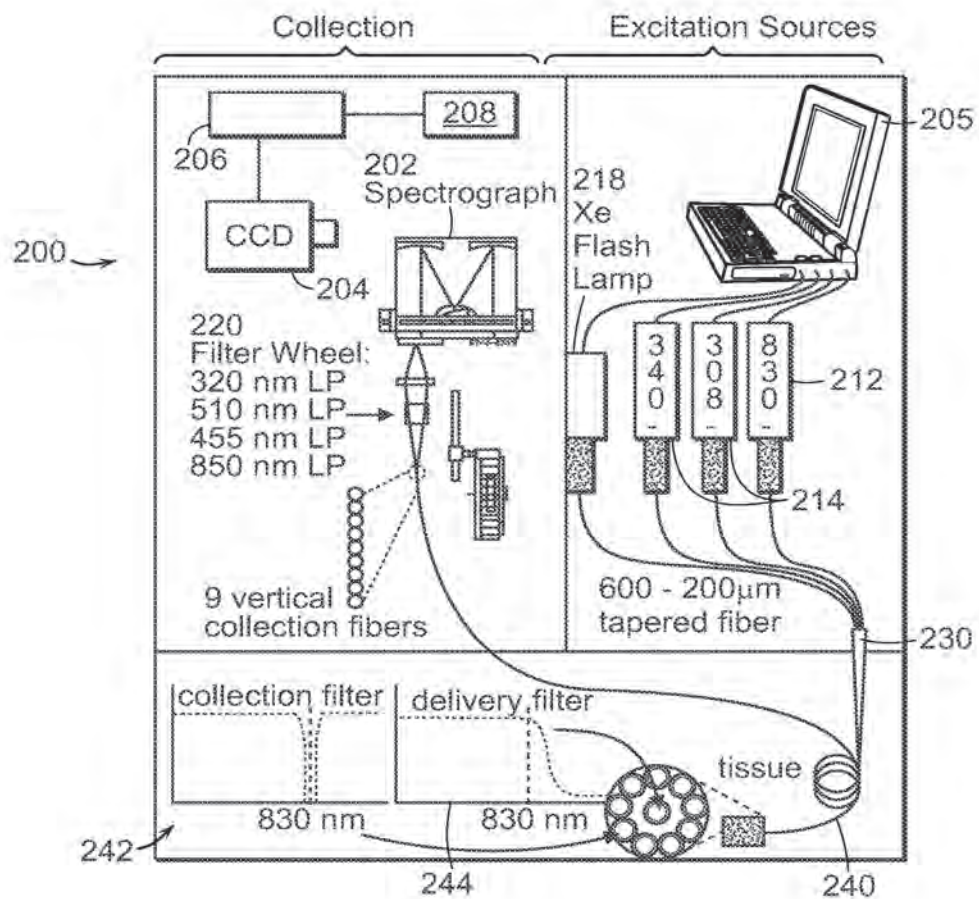


FIG. 14A

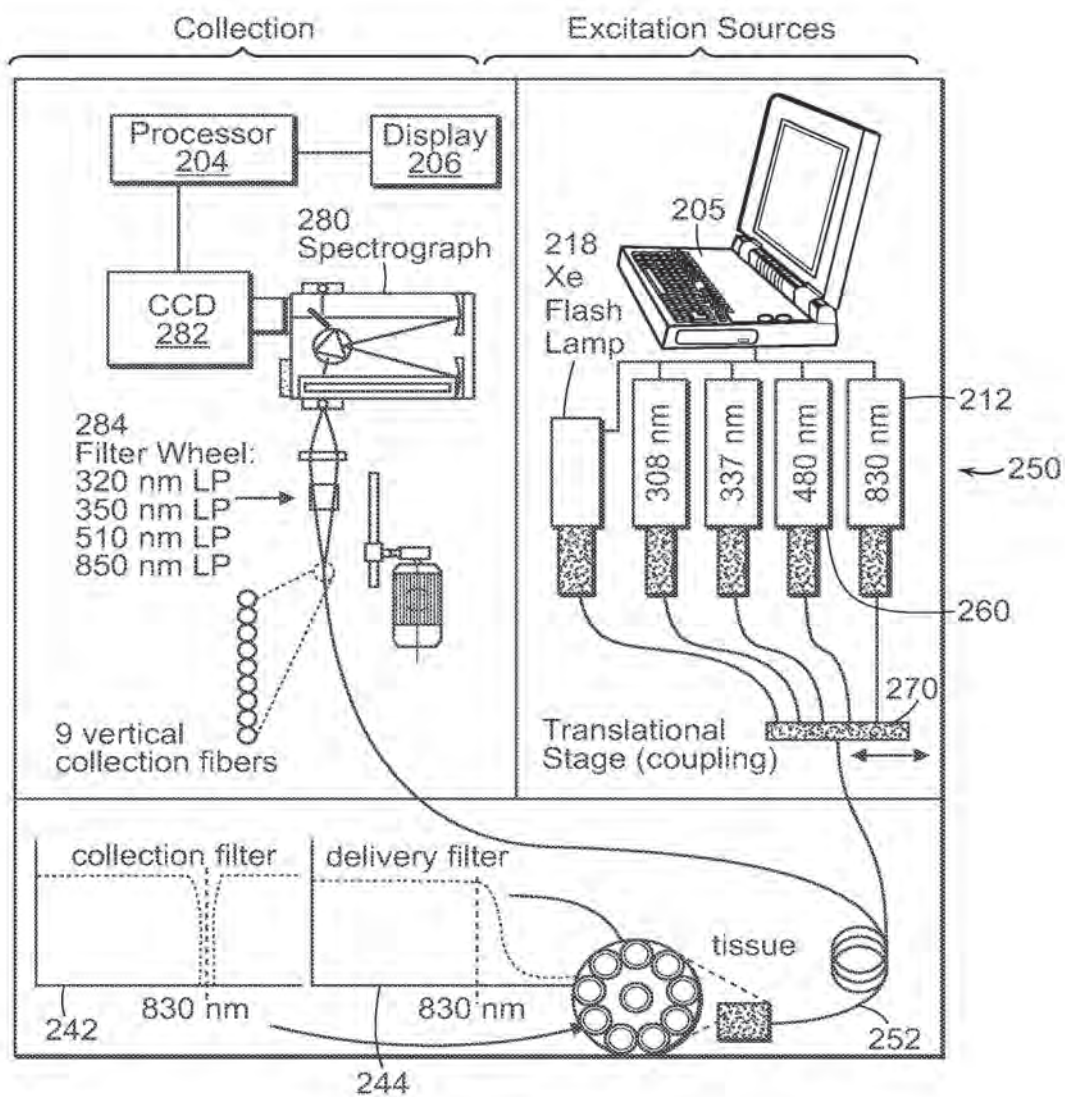


FIG. 14B

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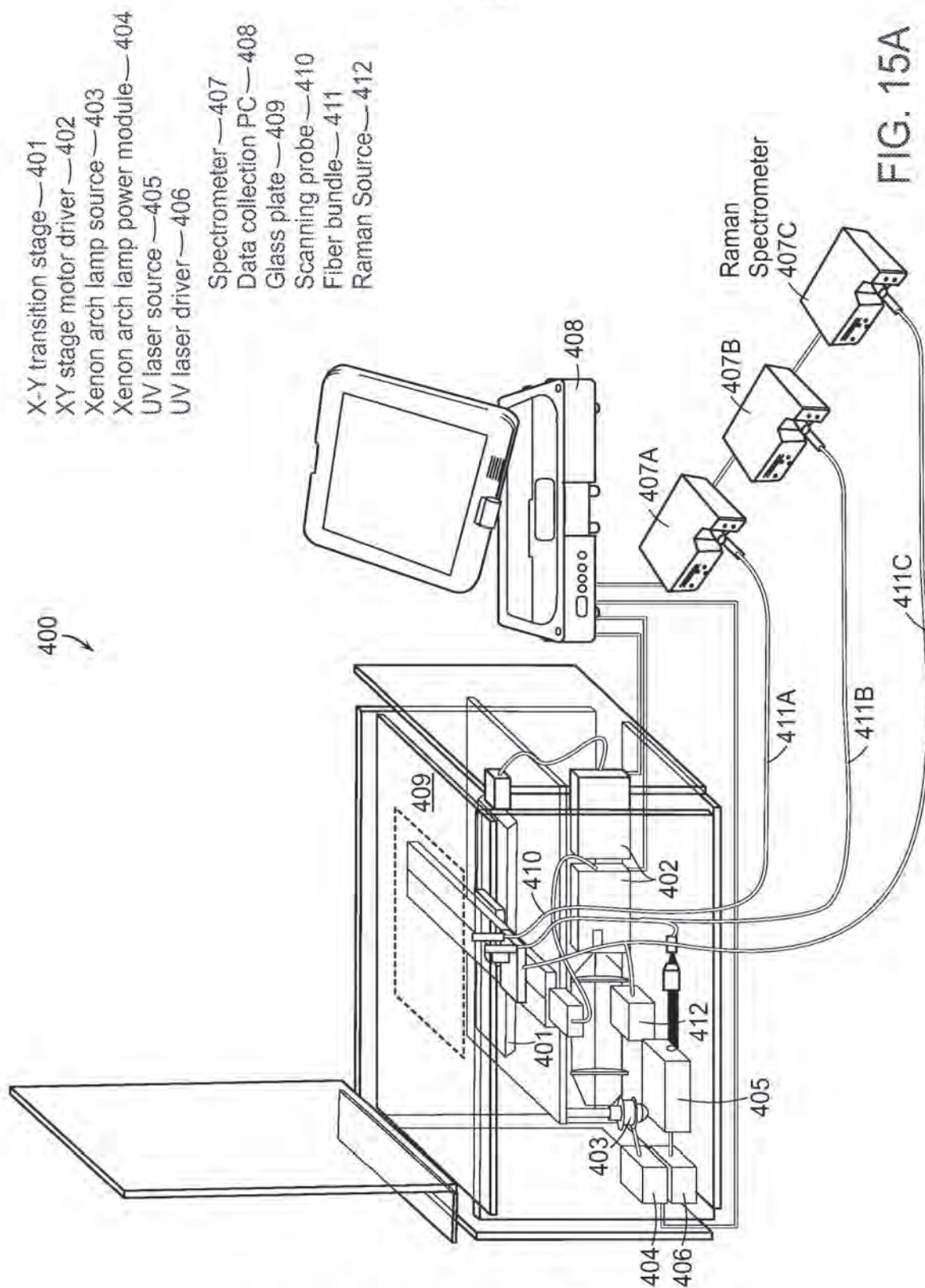


FIG. 15A

SUBSTITUTE SHEET (RULE 26)

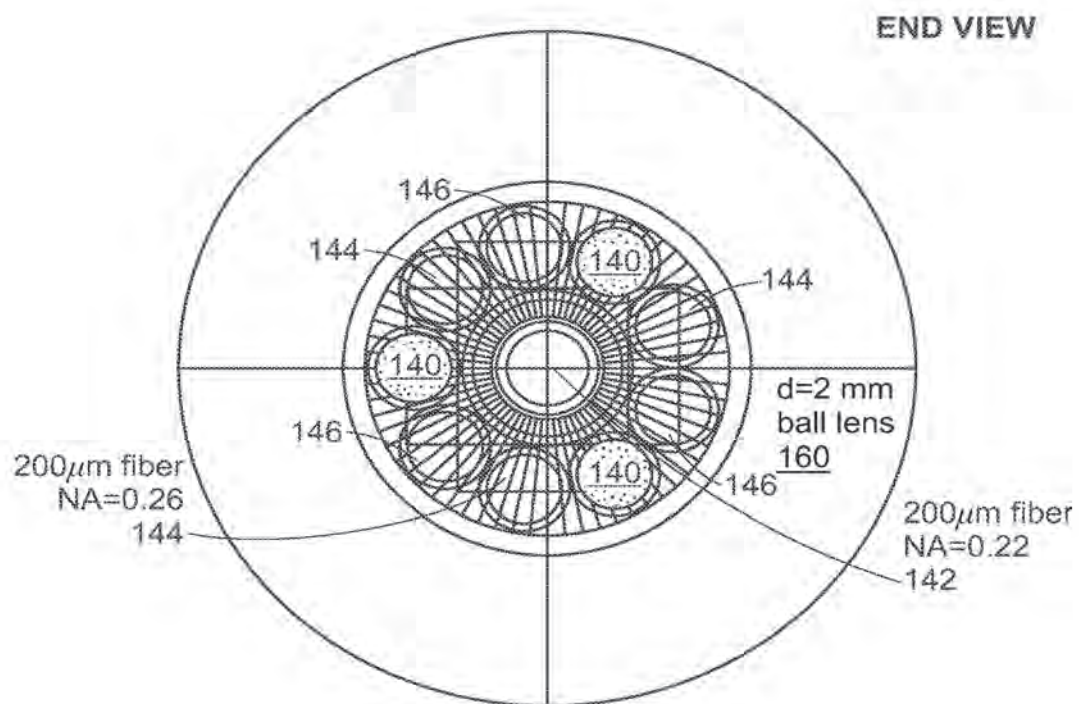
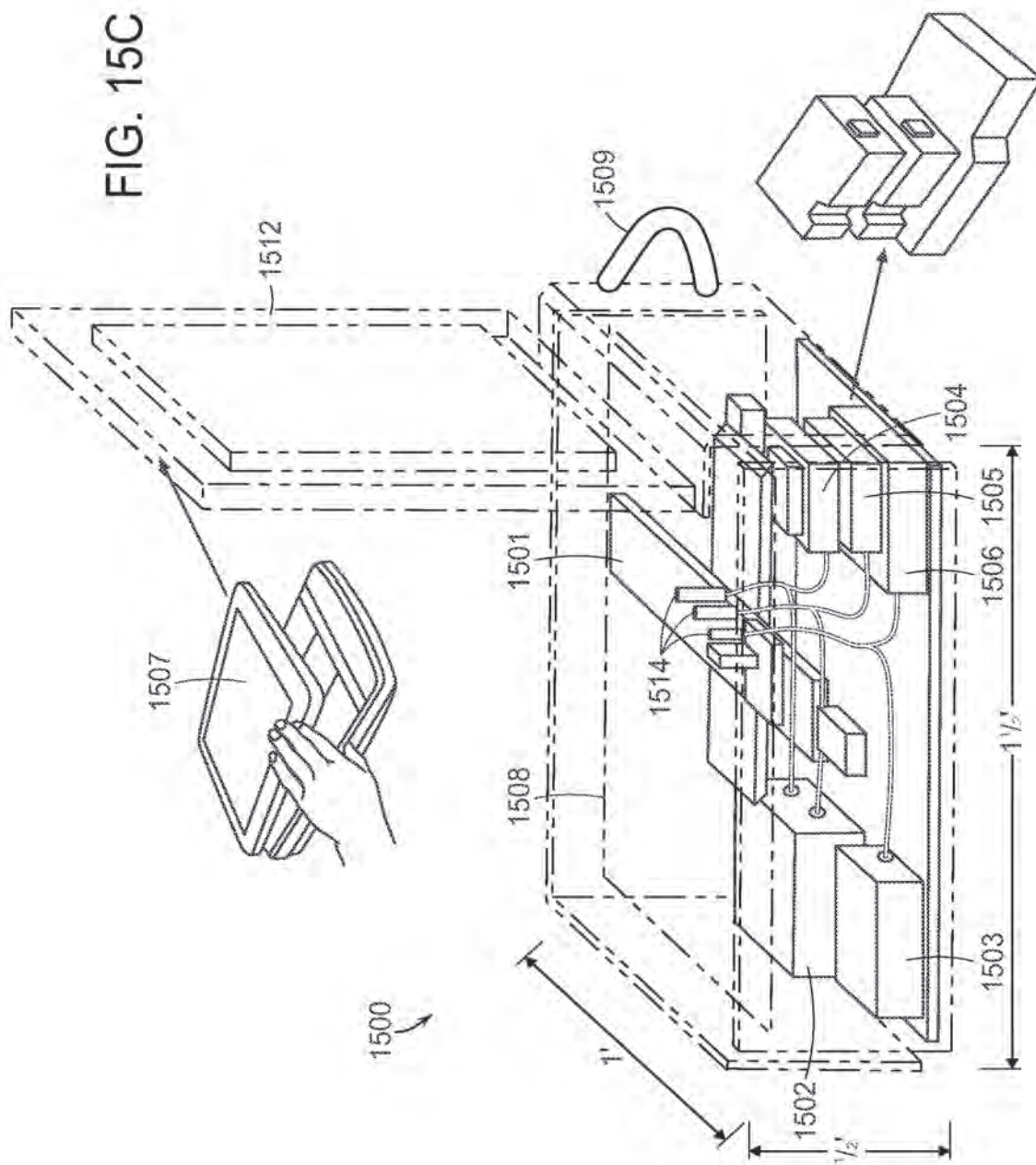


FIG. 15B

SUBSTITUTE SHEET (RULE 26)

FIG. 15C



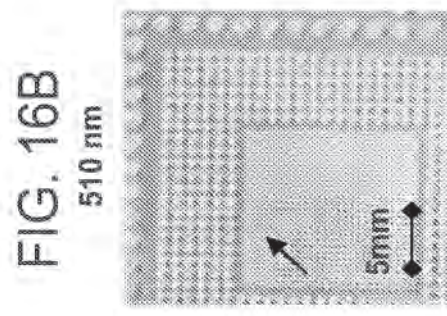
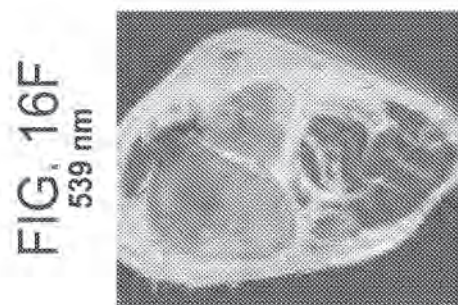
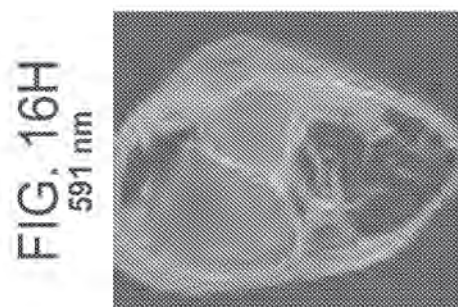
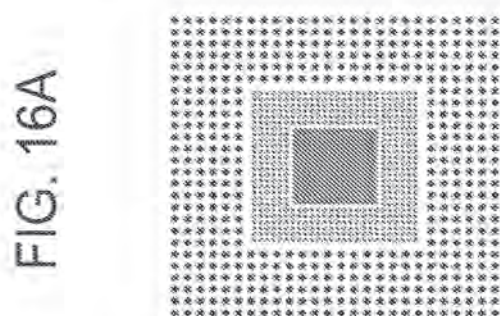
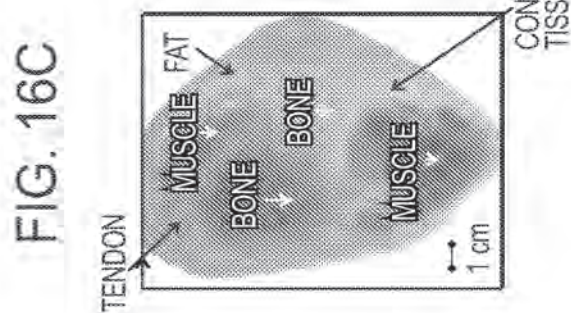
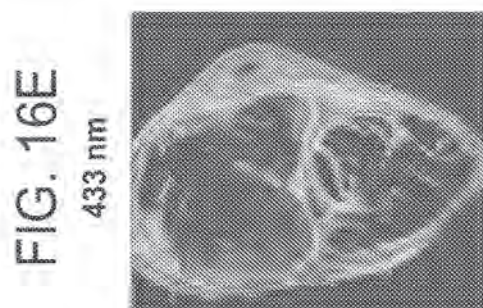
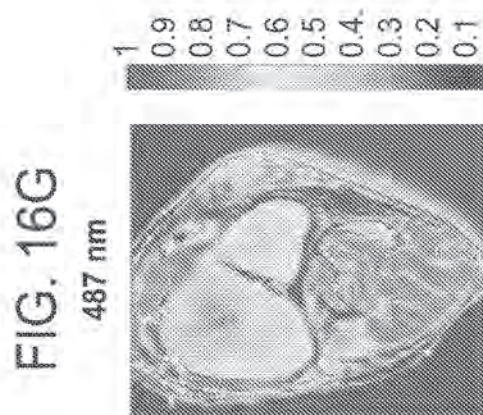


FIG. 17A

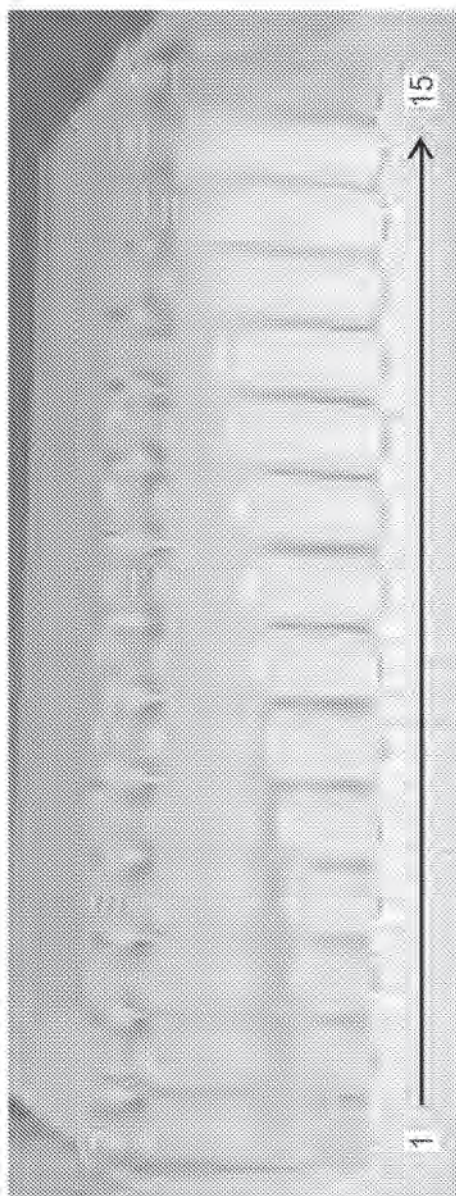


FIG. 17B

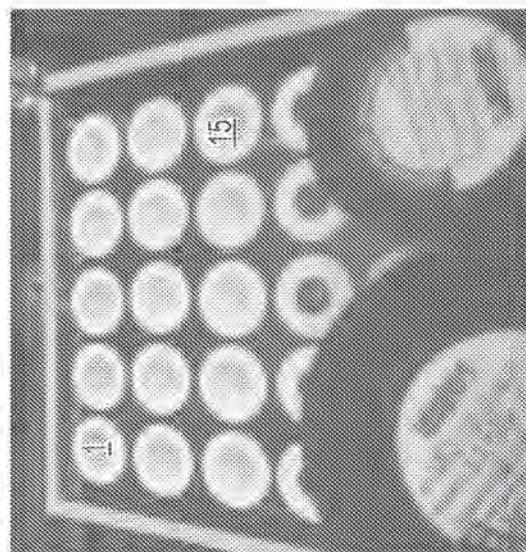
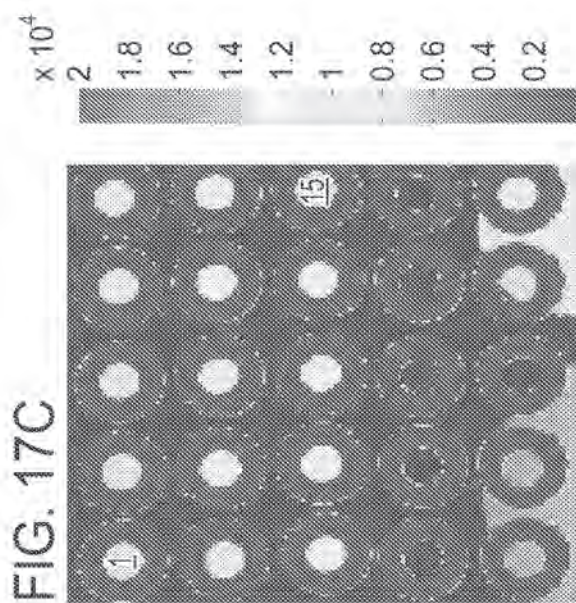


FIG. 17C



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FIG. 18A

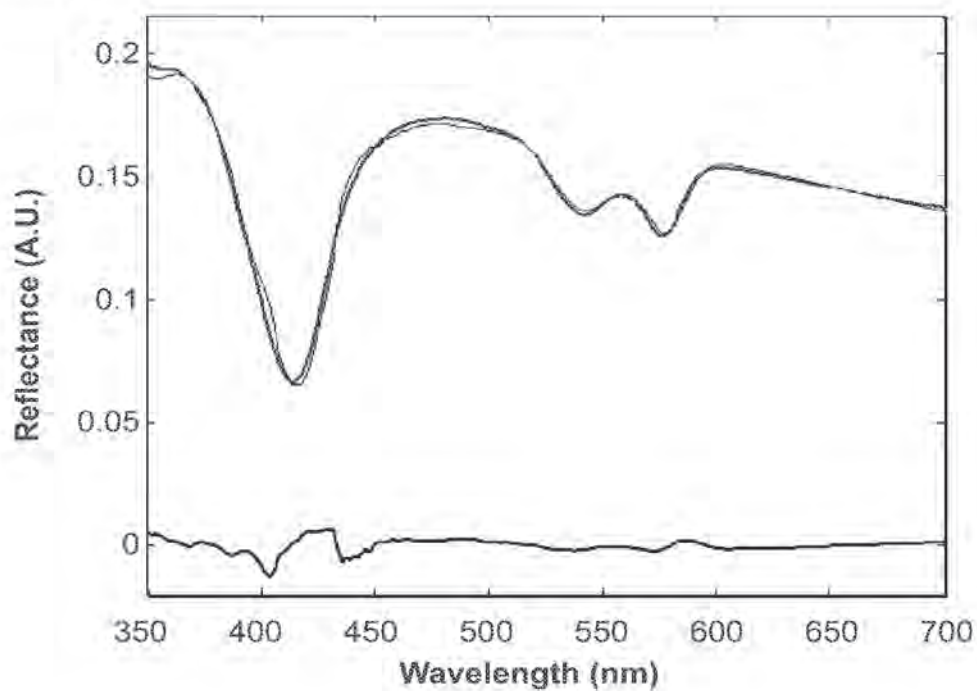
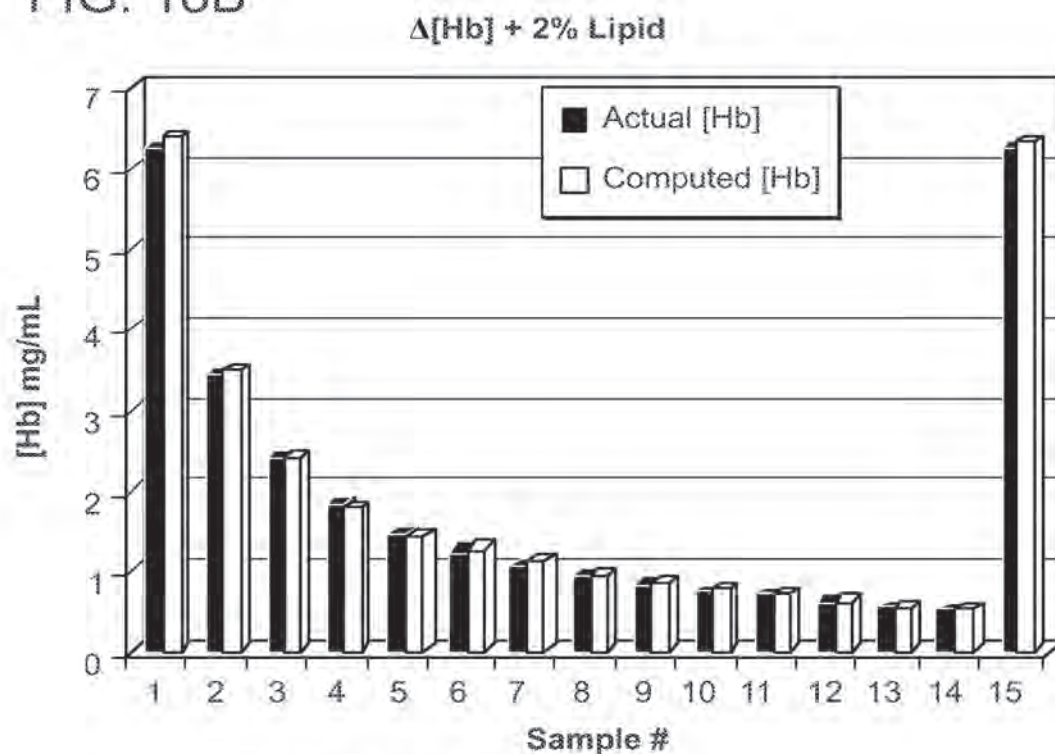


FIG. 18B



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FIG. 19A

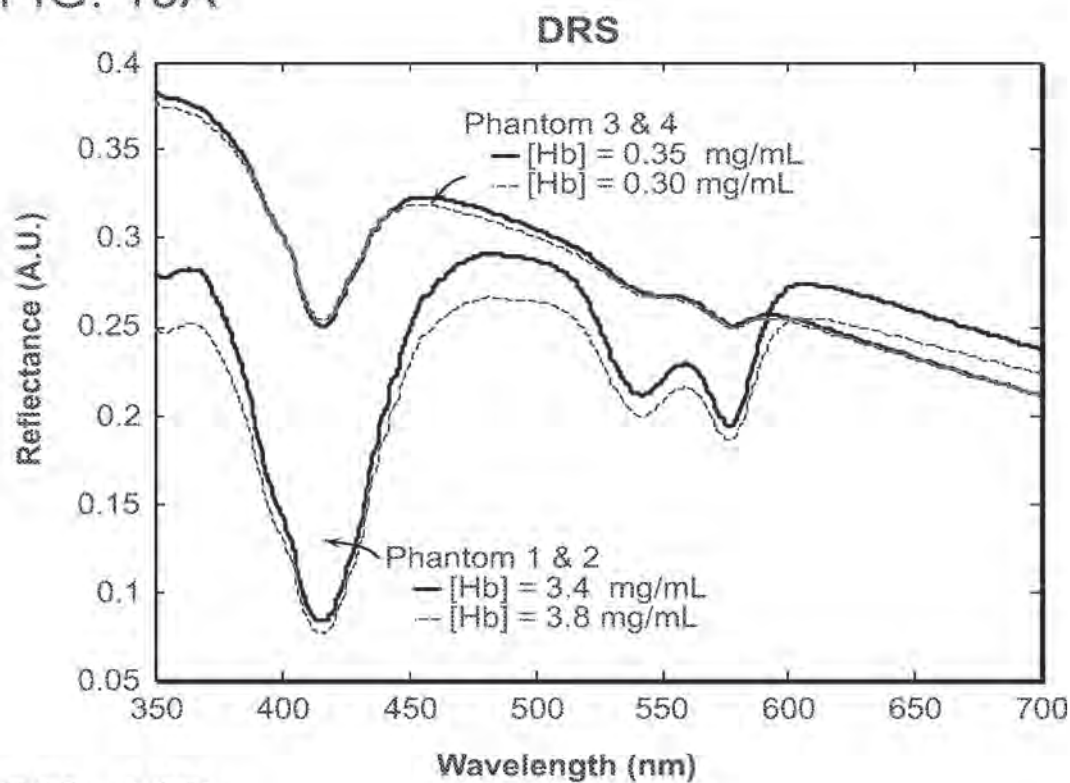
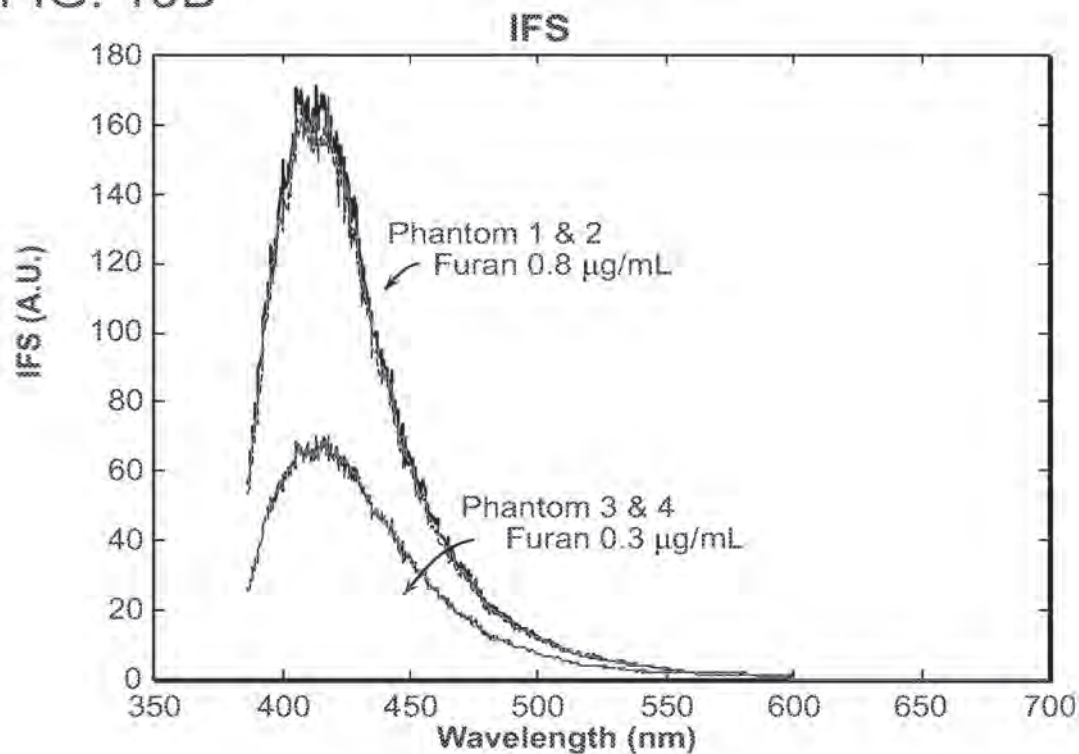
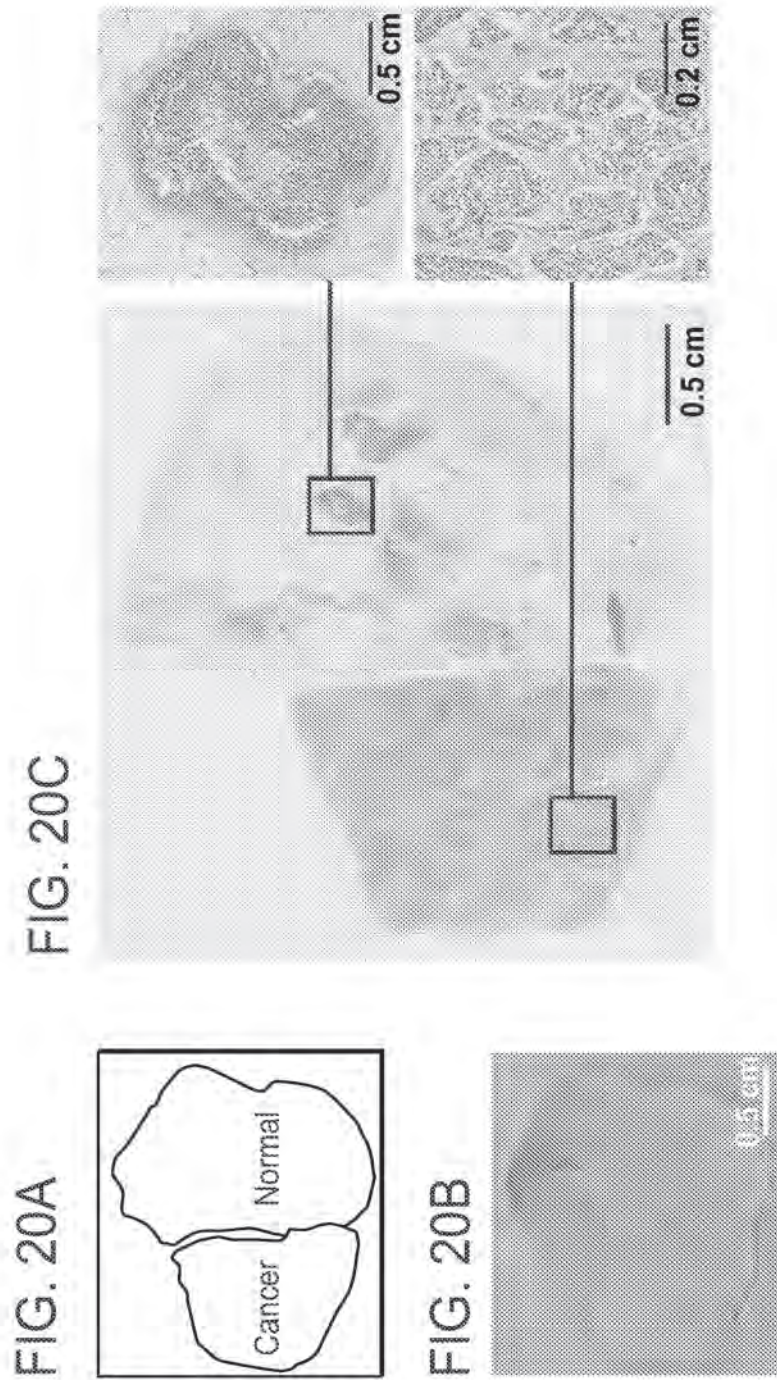


FIG. 19B



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FIG. 20D

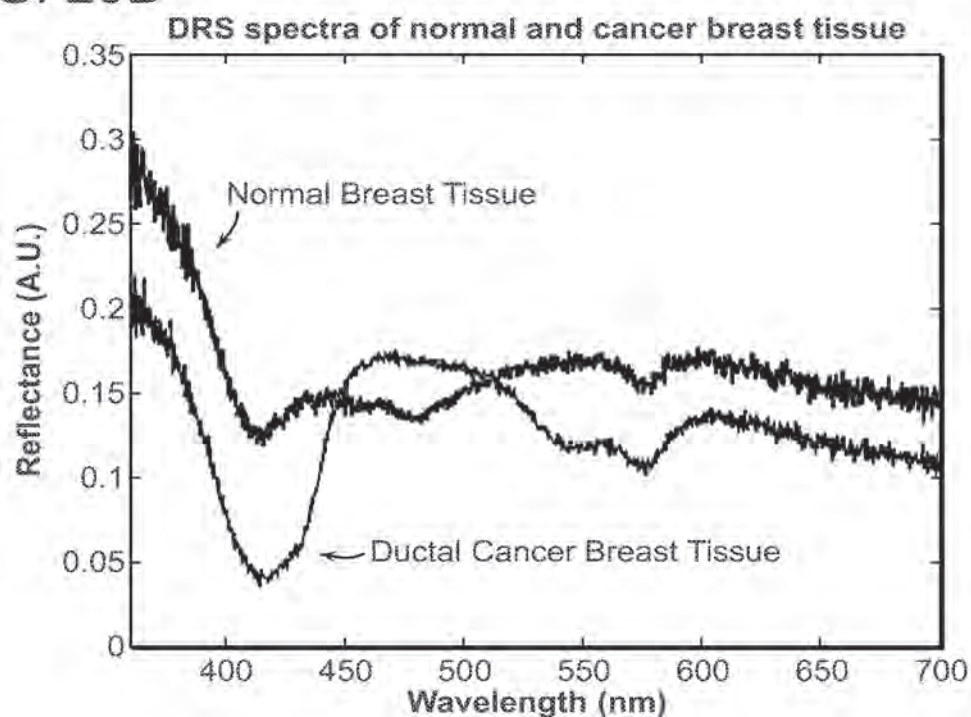
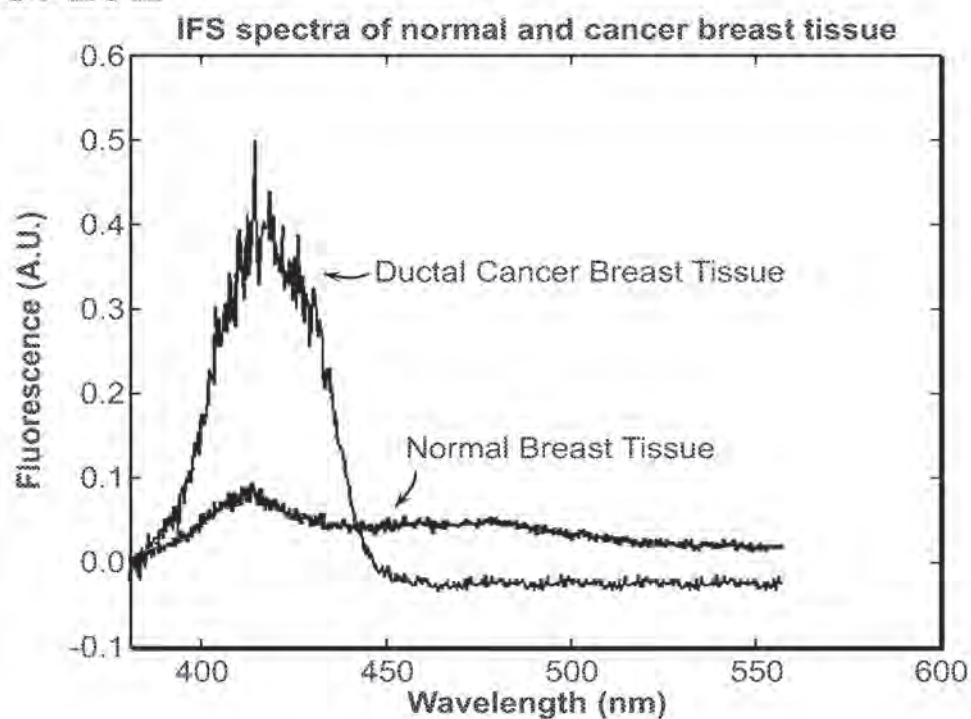
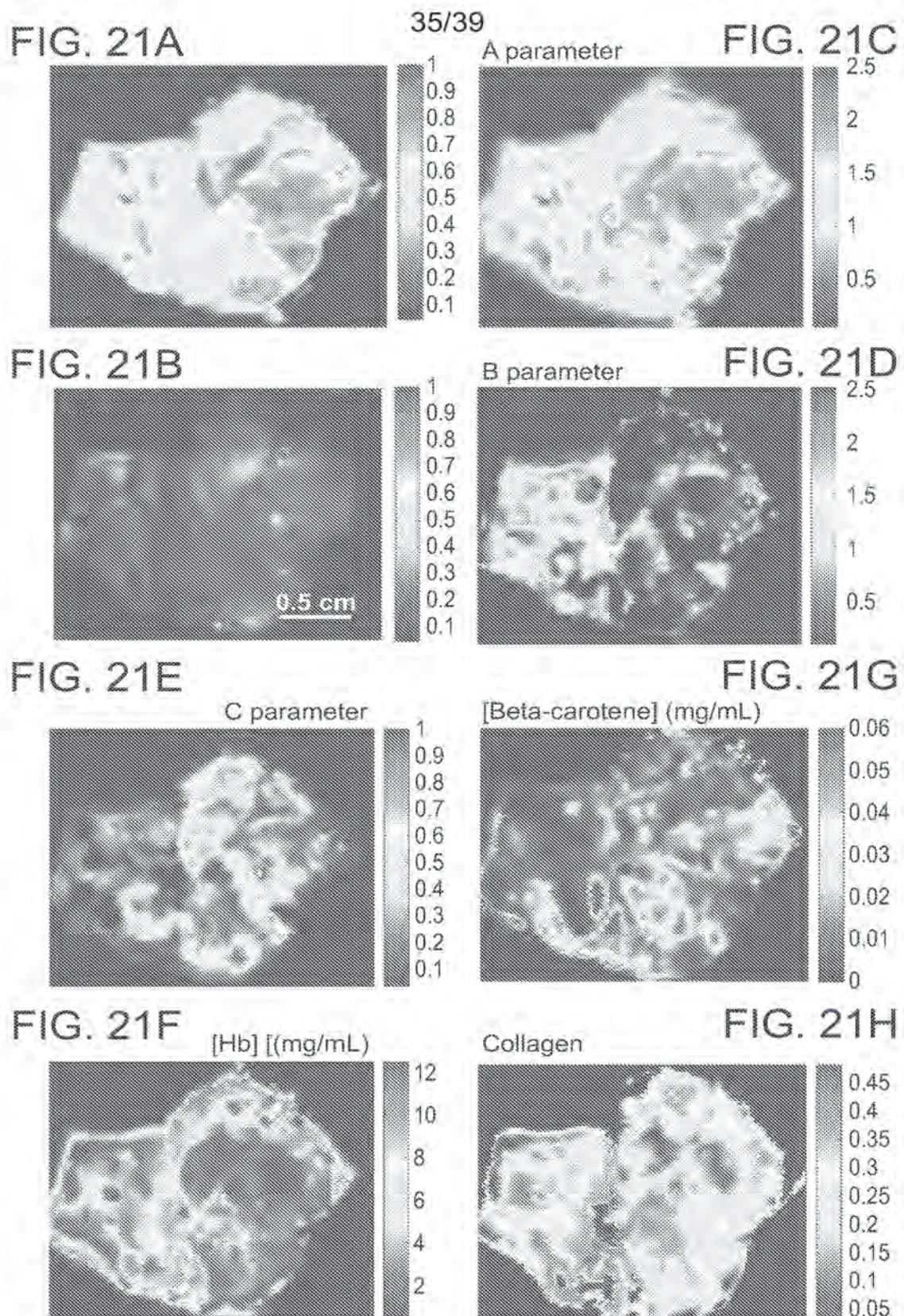


FIG. 20E



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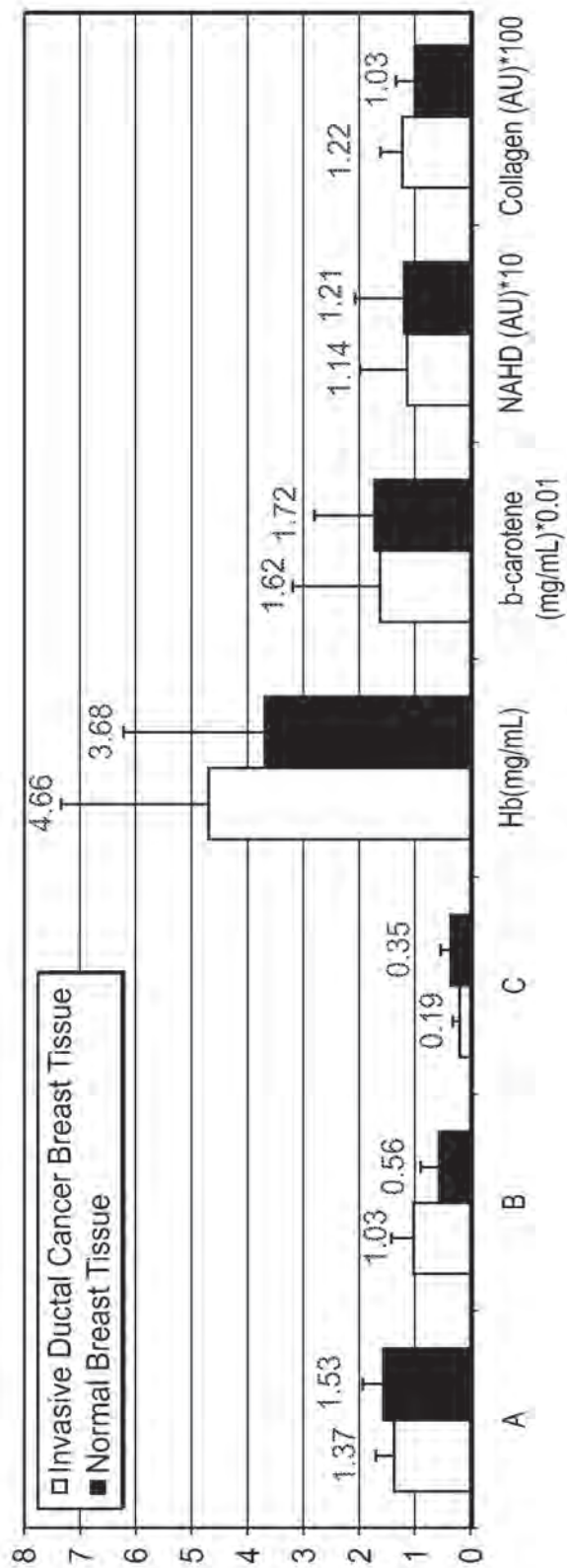


FIG. 22

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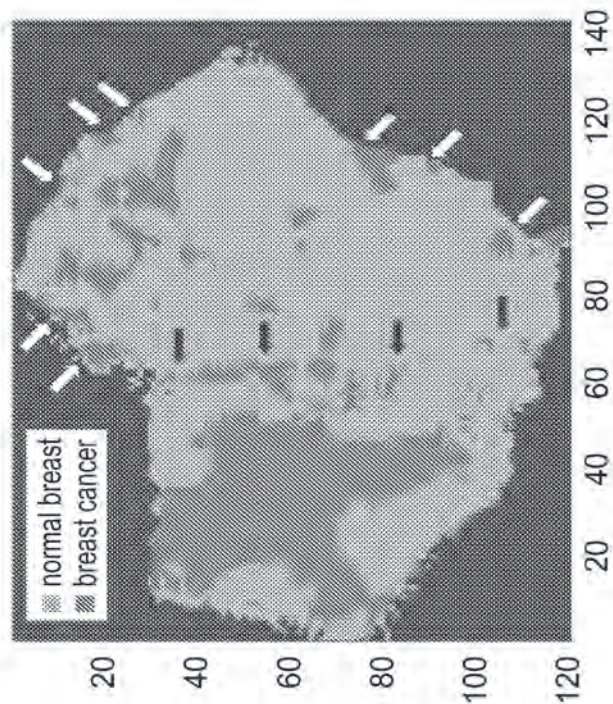


FIG. 23B

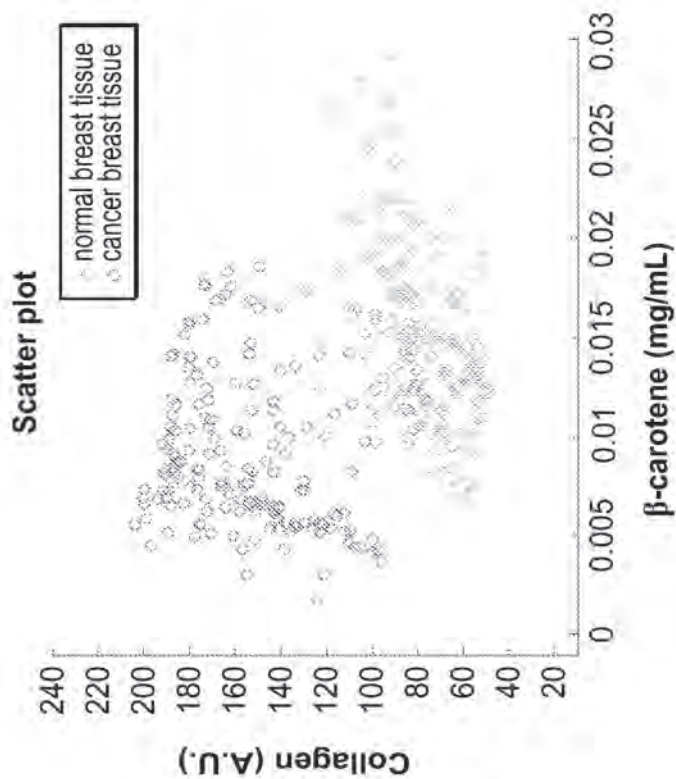
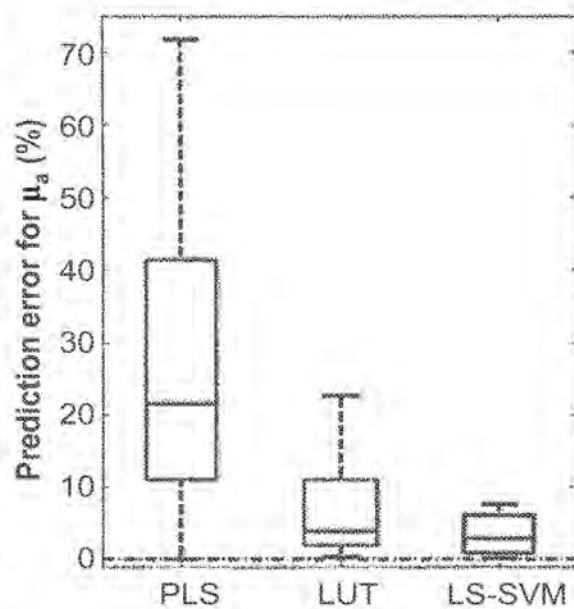
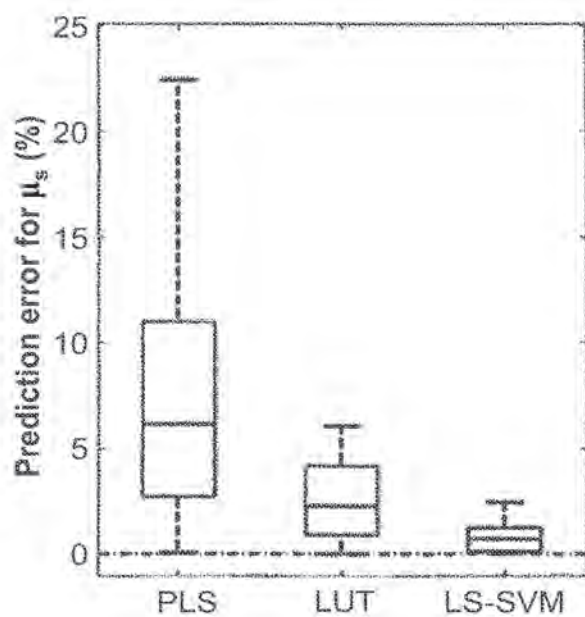
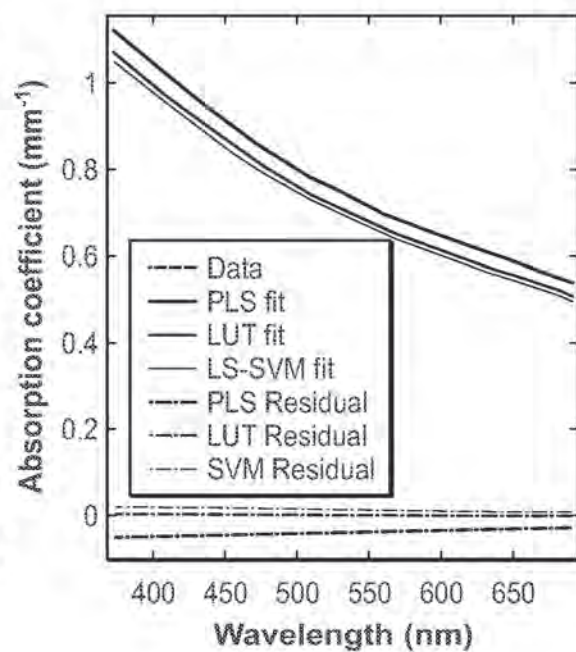
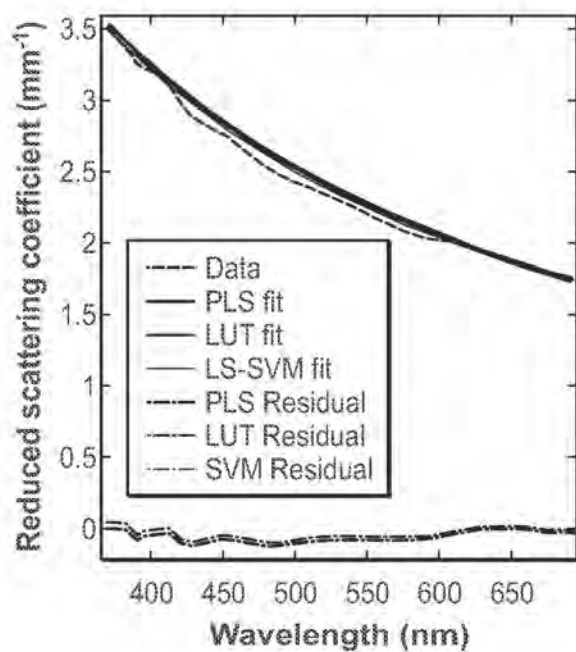


FIG. 23A

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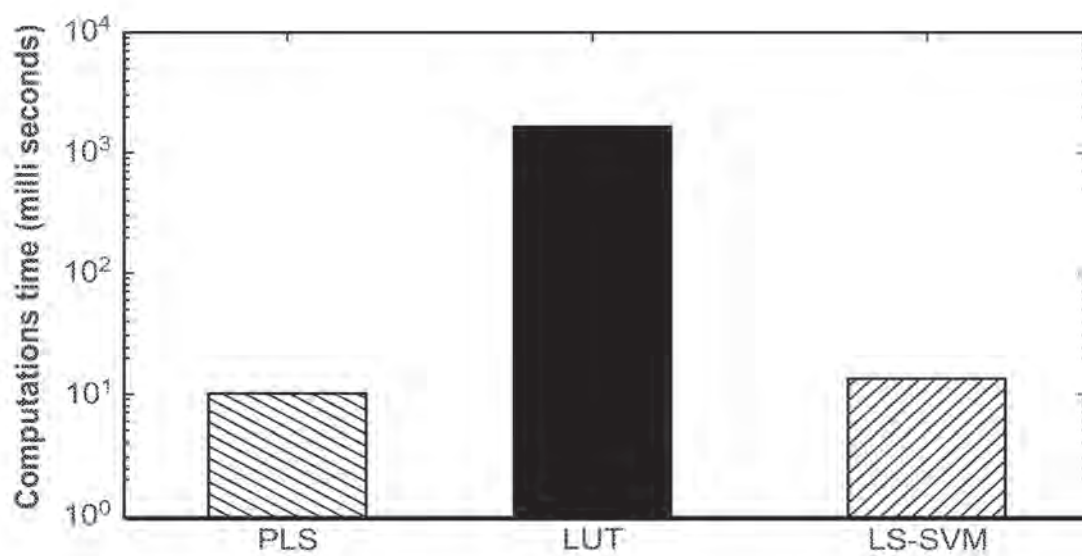


FIG. 26

SUBSTITUTE SHEET (RULE 26)

TRANSMITTAL FORM <i>(to be used for all correspondence after initial filing)</i>	Application Number	16/522,237-Conf. #4736
	Filing Date	July 25, 2019
	First Named Inventor	David Wilson
	Art Unit	1649
	Examiner Name	A. M. Weidner
Total Number of Pages in This Submission	Attorney Docket Number	Q0052.70018US03

ENCLOSURES (Check all that apply)		
☐ Fee Transmittal Form ☐ Fee Attached ☐ Amendment/Reply ☐ After Final ☐ Affidavits/declaration(s) ☐ Extension of Time Request ☐ Express Abandonment Request ☒ Information Disclosure Statement ☐ Certified Copy of Priority Document(s) ☐ Reply to Missing Parts/Incomplete Application ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53	☐ Drawing(s) ☐ Licensing-related Papers ☐ Petition ☐ Petition to Convert to a Provisional Application ☐ Power of Attorney, Revocation Change of Correspondence Address ☐ Terminal Disclaimer ☐ Request for Refund ☐ CD, Number of CD(s) _____ ☐ Landscape Table on CD	☐ After Allowance Communication to TC ☐ Appeal Communication to Board of Appeals and Interferences ☐ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) ☐ Proprietary Information ☐ Status Letter ☒ Other Enclosure(s) (please identify below): Form PTO-1449 Copies of cited references
Remarks		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT			
Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Michael J. Pomianek/		
Printed name	Michael J. Pomianek		
Date	February 8, 2021	Reg. No.	46,190

Certificate of Electronic Filing under 37 C.F.R. § 1.8	
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).	
Dated: February 8, 2021	Electronic Signature for Timothy Peng: /Timothy Peng/

DOCKET NO.: Q0052.70018US03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: David Wilson
Application No.: 16/522,237
Confirmation No.: 4736
Filed: July 25, 2019
For: METHODS OF DETERMINING A TREATMENT PROTOCOL FOR
AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A
BRAIN INJURY
Examiner: A. M. Weidner
Art Unit: 1649

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.8(a)(4).

Dated: February 8, 2021

Electronic Signature for Timothy Peng: /Timothy Peng/

MAIL STOP AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**STATEMENT FILED PURSUANT TO THE DUTY OF
DISCLOSURE UNDER 37 C.F.R. §§ 1.56, 1.97 AND 1.98**

Sir:

Pursuant to the duty of disclosure under 37 C.F.R. §§ 1.56, 1.97 and 1.98, the undersigned requests consideration of this Information Disclosure Statement.

PART I: Compliance with 37 C.F.R. § 1.97

This Information Disclosure Statement has been filed before the mailing of a first Office Action on the merits in the above-identified case.

No fee or certification is required.

PART II: Information Cited

The undersigned hereby makes of record in the above-identified application the information listed on the attached form PTO-1449 (modified PTO/SB/08). The order of presentation of the references should not be construed as an indication of the importance of the references.

8931143.1

The undersigned hereby makes the following additional information of record in the above-identified application.

The undersigned would like to bring to the Examiner's attention the following co-pending applications that may contain subject matter related to this application:

<u>Serial No.</u>	<u>Filing Date</u>	<u>Inventor(s)</u>	<u>Docket No.</u>
*16/906,257	06-19-2020	Duffy et al.	Q0052.70011US07
*17/135,848	12-28-2020	Rissin et al.	Q0052.70020US02
*15/550,625	08-11-2017	Pollock et al.	Q0052.70022US01
*16/648,788	03-19-2020	Wilson et al.	Q0052.70023US01
*16/616,134	11-22-2019	Díaz-Mochón et al.	Q0052.70024US01

*A copy of this reference is not provided as the Office has waived the requirement under 37 C.F.R. § 1.98(a)(2)(iii) for submitting a copy of a cited U.S. patent application if it is scanned to the Image File Wrapper system and is available on Private PAIR.

PART III: Remarks

Documents cited anywhere in the Information Disclosure Statement are enclosed unless otherwise indicated. It is respectfully requested that:

1. The Examiner consider completely the cited information, along with any other information, in reaching a determination concerning the patentability of the present claims;
2. The enclosed form PTO-1449 (modified PTO/SB/08) be signed by the Examiner to evidence that the cited information has been fully considered by the United States Patent and Trademark Office during the examination of this application;
3. The citations for the information be printed on any patent which issues from this application.

By submitting this Information Disclosure Statement, the undersigned makes no representation that a search has been performed, of the extent of any search performed, or that more relevant information does not exist.

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, in fact, prior art as defined by 35 U.S.C. § 102.

Notwithstanding any statements by the undersigned, the Examiner is urged to form his or her own conclusion regarding the relevance of the cited information.

An early and favorable action is hereby requested.

The Director is hereby authorized to charge any deficiency or credit any overpayment in the fees occasioned by the filing of this Information Disclosure Statement to our Deposit Account No. 23/2825 under Docket No. Q0052.70018US03 from which the undersigned is authorized to draw.

Respectfully submitted,

Electronic signature: /Michael J. Pomianek/
Michael J. Pomianek
Registration No.: 46,190
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Docket No.: Q0052.70018US03
Date: February 8, 2021



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/522,237	07/25/2019	David Wilson	Q0052.70018US03	4736
23628	7590	03/16/2021	EXAMINER	
WOLF GREENFIELD & SACKS, P.C. 600 ATLANTIC AVENUE BOSTON, MA 02210-2206			WEIDNER, ADAM M	
			ART UNIT	PAPER NUMBER
			1649	
			NOTIFICATION DATE	DELIVERY MODE
			03/16/2021	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Patents_eOfficeAction@WolfGreenfield.com
WGS_eOfficeAction@WolfGreenfield.com

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

DETAILED ACTION

This action is in response to claim amendments filed 1/8/20. Claims 11, 20-24, and 92-103 are pending and under examination.

Notice

Claim 92 is the independent claim. Claims must depend on a previous claim, which is not the case for claims, e.g., 11, which depends on claim 92. MPEP §608.01(n)(F) states that, in such case where the dependency is clear—as it is here—no objection to form will be made; the claims will be renumbered appropriately upon allowance. See also MPEP §608.01(n)(IV).

Information Disclosure Statement

The information disclosure statement filed 2/8/21 which has been struck through fails to comply with the provisions of 37 CFR 1.97, 1.98 and MPEP § 609 because it fails to provide a column for the examiner's initials next to each document (37 CFR 1.98(1)(ii)). It has been placed in the application file, but the information referred to therein has not been considered as to the merits. Applicant is advised that the date of any re-submission of any item of information contained in this information disclosure statement or the submission of any missing element(s) will be the date of submission for purposes of determining compliance with the requirements based on the time of filing the statement, including all certification requirements for statements under 37 CFR 1.97(e). See MPEP § 609.05(a).

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

The factual inquiries for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 11, 20-24, and 92-102 is/are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Duffy (US 20100075407; cited on page 9 of IDS 1/13/20) in view of Quanterix 2010 IQT Technology Focus Day (hereinafter Quanterix 2010a; cited on IDS 1/13/20 p. 16).

Regarding claim 92, Duffy teaches:

- Obtaining a volume of body fluid comprising blood, plasma, or serum: fluid samples may come from human blood, serum, or plasma; paragraph 144.

- Diluting the volume of body fluid: the dynamic range of the assay may be increased by diluting the sample; paragraphs 213 and 222.
- Quantifying through the use of an analytical protein concentration measurement assay a concentration of protein in the volume of body fluid to produce the body fluid sample: the whole of Duffy is directed to the use of such an assay to measure the protein; see, e.g., the claims such as claims 1, 8, 64, 82, and 111.
- Where the limit of detection of the assay is less than about 0.2 pg/mL: Duffy does not explicitly state the limit of detection. However, the steps in the method of Duffy meet all the criteria for the instantly claimed steps and Duffy characterizes the assay as "Ultra-Sensitive" (title). Thus, absent evidence to the contrary, the method of Duffy meets the limit of quantification requirements.

Duffy does not teach the protein being detected is tau. While occurring solely in the preamble, the claim is clearly limited to a sample obtained from a patient recovering from a brain injury, and Duffy does not teach such a patient.

Nevertheless, one of ordinary skill in the art at the time of the invention would have found it obvious that the method of Duffy could be used to detect less than about 5 pg/mL in a blood/serum/plasma sample of a patient recovering from brain injury. As noted by the United States Supreme Court, if a person of ordinary skill can implement a predictable variation, § 103 likely bars its patentability. For the same reason, if a technique has been used to improve one method (i.e., Duffy's ultra-sensitive assay for measuring proteins in blood), and a person of ordinary skill would recognize that it would improve similar methods (i.e., measuring specifically tau protein in those recovering from brain injury) in the same way, using the technique is obvious unless its actual application is beyond his or her skill. KSR, 127 S. Ct. at 1740. "When there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product is not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try might show it was obvious under 35 U.S.C. 103." KSR Int'l Co. v. Teleflex Inc., 127 S.Ct. 1727, 1742, 82USPQ2d 1385, 1396 (2007).

Quanterix 2010a teaches using SiMoA (single molecule array; p.1) which is the same assay as Duffy (see e.g., Duffy title). Whereas Duffy broadly teaches the assay is suitable to detecting any protein of interest in blood, Quanterix 2010a explicitly teaches the assay can detect tau in a blood sample (p.22) and suggests doing so in a subject recovering from a brain injury; p.29 teaches using the assay to detect tau in a subject who has suffered—and therefore is recovering from—TBI (traumatic brain injury). Quanterix 2010a also makes clear that the assay detects single molecules (p.5) and detection in the aM range (p. 4, 10, 11, 14). Thus, the Quanterix 2010a assay clearly has a detection limit at or below "about 0.2 pg/mL", providing further evidence that the Duffy assay does as well. Moreover, this provides the predictable and expected result of being able to detect tau in a body fluid sample less than about 5 pg/mL. Note that this amount in the body fluid is entirely dependent on the subject and the endogenous levels and not an active step or manipulation of the claimed method. As one of ordinary skill in the art would have expected to the method to detect such levels, detecting such levels in a sample where this is the endogenous level would have been obvious.

Regarding claims 11, 21, 22, 23, and 24, the nature of the brain injury would not affect the sensitivity of the assay nor its ability to detect tau in the blood. In the same way, it would have been a predictable variation of the disclosure to measure tau in any sample suspected of containing tau, particularly as Quanterix 2010a teaches there is theoretically no limit on the detection sensitivity (p.10) and that practically levels of attamolar concentrations have been detected (p. 11). Quanterix 2010a teaches the assay detects tau in blood of healthy controls (p.22) and thus one of ordinary skill in the art at the time of the invention would have had a reasonable expectation of success when using the method to measure tau in any subject irrespective of that subject's health/disease, including those suffering a hypoxic event, cardiac arrest, stroke, etc, as one could predictably produce a body fluid sample and detect tau.

Regarding claim 20, the steps are the same and therefore the limits of detection are the same. Further, Quanterix 2010a teaches a practical LOD in the attamolar range (p.11) and no theoretical floor to the LOD (p.10); this meets the limitations and makes obvious a limit of detection less than about 0.02 pg/mL.

Regarding claim 93:

- Exposing a plurality of capture objects that each include a binding surface having affinity for tau protein molecules to the volume of body fluid: Duffy teaches the analyte is detected by exposing a plurality of capture objects (components) to a sample (fluid sample; see above) such that the two associate (bind); see paragraph 7. Duffy also teaches where these capture components comprise a binding surface for the analyte (paragraph 94). Quanterix 2010a teaches the same method (see above) where the capture object contains an antibody (binding surface) for the analyte (p.13) and wherein the analyte is tau (see above). Thus, it would have been a predictable variation of Duffy to select specifically a tau binding surface when set to measure tau.
- Immobilizing at least a portion of the tau protein molecules with respect to the plurality of capture objects such that at least some of the capture objects associate with at least one tau protein molecule and a statistically significant fraction of the capture objects do not associate with any tau protein molecules: this is how the assays of both Duffy and Quanterix 2010a function. See for example Quanterix 2010a p.5, 9, and 13 and Duffy paragraphs 185 and 199.
- Determining a proportion of the plurality of capture objects that associated with a tau protein molecule in step ii: as above, this is how the assays of both Duffy and Quanterix 2010a function. See Quanterix 2010a p.5, 9, and 13 and Duffy paragraphs 185, and 197-198.
- Determining a measure of tau protein molecule concentration in the volume of body fluid based at least in part on the proportion determined in step iii: both Duffy and Quanterix 2010a teach using the method to determine the concentration (a measure) of the protein while Quanterix 2010a makes obvious that this protein is tau. See also Duffy paragraph 197-198 and Quanterix 2010a p.22.

Regarding claim 94, Quanterix 2010a teaches the capture object is a bead (p.13) as does Duffy (paragraphs 226, 257, 258, 264, and claim 9).

Regarding claim 95, these limitations are taught by both documents. For example, Quanterix 2010a p.5 showing a plurality of wells (spatially segregated in a plurality of locations) where each well contains

either 1 (statistically significant fraction of locations contain a single tau protein) or 0 (statistically significant fraction of locations do not contain any tau protein) molecules, the molecule being tau as discussed above. See also Duffy claim 188 and paragraph 197.

Regarding claim 96, Quanterix 2010a teaches the locations are reaction vessels (p.5) as does Duffy (e.g., paragraph 197).

Regarding claim 97, Quanterix 2010a teaches measurement of total tau in a human blood sample and thus necessarily includes measurement of the recited isoforms of tau. Further, as above, Duffy teaches the method is broadly applicable to any analyte of interest and Quanterix 2010a teaches tau, making it obvious to one of ordinary skill in the art that the method could be used to detect known isoforms of tau. Finally, a generic disclosure ("measure tau" as taught by Quanterix 2010a) can still anticipate (and thus make obvious) a species within that genus (the tau isoforms) where a person of the art could immediately envisage the species of that genus. The instant claim includes well-known isoforms of tau which could be immediately envisaged by the skilled artisan and therefore detecting these isoforms would have been obvious. Selection of a specific tau isoform to include would have been a predictable variation of the disclosure of Quanterix 2010a given the robust nature of the technology to accurately measure tau and a variety of other proteins with such sensitivity.

Regarding claim 98, as above, Quanterix 2010a teaches measuring total tau, which will necessarily include "at least a portion of the tau protein molecules are phosphorylated".

Regarding claims 99-101, it would have been obvious to use blood, plasma, or serum samples as discussed above.

Regarding claim 102, a blood/plasma/serum sample does not contain CSF.

Therefore, claims 11, 20-24, and 92-102 would have been obvious.

Claims 11, 20-24, and 92-103 is/are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Duffy (US 20100075407; cited on page 9 of IDS 1/13/20) in view of Quanterix 2010 IQT Technology Focus Day (hereinafter Quanterix 2010a; cited on IDS 1/13/20 p. 16) and further in view of Wunderlich (cited on p. 28 of IDS 1/13/20).

Claims 11, 20-24, and 92-102 would have been obvious as above.

Regarding claim 103, Duffy/Quanterix 2010a does not teach that tau is present in a blood sample within 12 hours of a brain injury. However, Quanterix 2010a teaches tau is present and detectable in the blood samples of an otherwise healthy subject ("disease-free" p.22), so regardless of the time relative to an injury, one of ordinary skill in the art would have a reasonable expectation of detecting tau in that sample. Further, Wunderlich teaches that approximately 30 pg/mL of tau are detected in blood at 3, 6, and 12 hours after a stroke (brain injury; see figure 1b and p.559 C1). Thus, Quanterix 2010a provides the teachings to make obvious measuring tau in the blood of a subject with a brain injury while Wunderlich provides a reasonable expectation of detecting tau in said blood within 12 hours of a brain injury, making such a method of "producing a body fluid sample" obvious.

Regarding claim 98, measuring total tau includes measuring at least one phosphorylated tau molecule ("a portion") as discussed above. However, Wunderlich also makes this explicit, using antibodies which measure total tau which is defined as "normal tau and phosphorylated tau" (p.559 C1).

Regarding claims 11 and 22-23, Wunderlich teaches tau serum levels in a patient who has suffered (and is thus recovering from) acute ischemic stroke (table 1), which is a hypoxic event.

Regarding claim 24, Wunderlich teaches performing thrombolysis on the patient's with ischemic stroke (p.560 C1; table 1). Thus, Wunderlich clearly includes thrombotic stroke (where the hypoxic event is caused by a thrombosis). Further, thrombosis causing such hypoxic events is well known and one of ordinary skill in the art at the time of the invention would have had a reasonable expectation that the findings of Wunderlich could be predictably applied to such subject, particularly as Wunderlich notes that thrombolysis did not significantly lower tau values.

Therefore, claims 11, 20-24, and 92-103 would have been obvious.

Claims 11, 20-24, and 92-102 is/are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Duffy (US 20100075407; cited on page 9 of IDS 1/13/20) in view of Quanterix 2010 IQT Technology Focus Day (hereinafter Quanterix 2010a; cited on IDS 1/13/20 p. 16) in further in view of Mortberg (cited on p. 22 of IDS 1/13/20).

Claims 11, 20-24, and 92-102 would have been obvious as above.

Regarding claim 21, Mortberg teaches measuring tau in plasma after cardiac arrest at 2, 6, 12,

24, 48, and 96 hours (p.28; figure 18), detecting significant levels after 48 hours. Thus, one of ordinary skill in the art at the time of the invention would have found it obvious to utilize the method of Duffy/Quanterix 2010a with a reasonable expectation of success. One of ordinary skill in the art would have had a reasonable expectation of detecting tau in blood where any tau existed for the reasons above, and Mortberg confirms there is tau to be detected after a cardiac arrest. While the 48/96 hour samples had more than the instantly claimed 5 pg/ml, it is noted that the tau present in the blood of these cardiac arrest patients was clearly below the limit of detection for the method of Mortberg, a problem which is solved by Duffy/Quanterix 2010a. Mortberg teaches the limit of detection is ~30 ng/L (30 pg/mL; p.28). Further evidence that the method of Mortberg alone simply did not detect tau in samples containing less than about 5 pg/mL is that no tau was detected by the method of Mortberg until 24-48 hours after cardiac arrest, when one of ordinary skill in the art was well aware that tau existed in such samples, given the teachings of e.g., Quanterix 2010a that tau exists in blood samples of "disease-free" controls. Thus, taking the documents in combination, one of ordinary skill in the art would have found it obvious to use the superior detection limits in a method such as Duffy/Quanterix 2010a to measure tau at less than ~5 pg/mL in a subject recovering from cardiac arrest, producing a sample as instantly claimed with a reasonable expectation of success.

Regarding claim 11, cardiac arrest is a hypoxic event.

Regarding claim 23, cardiac arrest is an ischemic event.

Therefore, claims 11, 20-24, and 92-102 would have been obvious.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the

reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP § 2146 *et seq.* for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-l.jsp.

Claim 11, 20-24, and 92-103 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 11, 24-30, 100-102, 104, 107-114, 132-133, and 135 of copending Application No. 14/111,331 (reference application) in view of Quanterix 2010a. Although the claims at issue are not identical, they are not patentably distinct from each other because the reference document is directed to the same assay (e.g., claim 109, detection limit less than 0.2 pg/mL; plurality of samples within 24 hours of a hypoxic brain injury, claim 133). The difference is in the reference application being drawn to detecting beta amyloid rather than tau. However, Quanterix 2010a makes clear that the method is applicable to detecting tau.

Claim 11, 20-24, and 92-103 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-25 of U.S. Patent No. 10393759. Although the claims at issue are not identical, they are not patentably distinct from each other because the reference method detects tau in the same samples in the same way, differing only in that the reference claims then utilize this gathered data. Thus, the reference claims anticipate the instant method.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ADAM M WEIDNER whose telephone number is (571)272-3045. The examiner can normally be reached on M-F 9-16.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Stucker can be reached on 571-272-0911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <https://ppair-my.uspto.gov/pair/PrivatePair>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Adam Weidner/
Primary Examiner, Art Unit 1649

Search Notes 	Application/Control No. 16/522,237	Applicant(s)/Patent Under Reexamination Wilson et al.
	Examiner ADAM M WEIDNER	Art Unit 1649

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
inventor/assignee east	03/11/2021	amw
review prosecution of parent 15/269142 and 14/111326	03/11/2021	amw

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

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DOCKET NO.: Q0052.70018US03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: David Wilson
Application No.: 16/522,237
Confirmation No.: 4736
Filed: July 25, 2019
For: METHODS OF DETERMINING A TREATMENT PROTOCOL FOR
AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A
BRAIN INJURY
Examiner: A. M. Weidner
Art Unit: 1649

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(b)(4).

Dated: February 8, 2021

Electronic Signature for Timothy Peng /Timothy Peng/

MAIL STOP AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**STATEMENT FILED PURSUANT TO THE DUTY OF
DISCLOSURE UNDER 37 C.F.R. §§ 1.56, 1.97 AND 1.98**

Sir:

Pursuant to the duty of disclosure under 37 C.F.R. §§ 1.56, 1.97 and 1.98, the undersigned requests consideration of this Information Disclosure Statement.

PART I: Compliance with 37 C.F.R. § 1.97

This Information Disclosure Statement has been filed before the mailing of a first Office Action on the merits in the above-identified case.

No fee or certification is required.

PART II: Information Cited

The undersigned hereby makes of record in the above-identified application the information listed on the attached form PTO-1449 (modified PTO/SB/08). The order of presentation of the references should not be construed as an indication of the importance of the references.

8931143.1

The undersigned hereby makes the following additional information of record in the above-identified application.

The undersigned would like to bring to the Examiner's attention the following co-pending applications that may contain subject matter related to this application:

<u>Serial No.</u>	<u>Filing Date</u>	<u>Inventor(s)</u>	<u>Docket No.</u>
*16/906,257	06-19-2020	Duffy et al.	Q0052.70011US07
*17/135,848	12-28-2020	Rissin et al.	Q0052.70020US02
*15/550,625	08-11-2017	Pollock et al.	Q0052.70022US01
*16/648,788	03-19-2020	Wilson et al.	Q0052.70023US01
*16/616,134	11-22-2019	Díaz-Mochón et al.	Q0052.70024US01

*A copy of this reference is not provided as the Office has waived the requirement under 37 C.F.R. § 1.98(a)(2)(iii) for submitting a copy of a cited U.S. patent application if it is scanned to the Image File Wrapper system and is available on Private PAIR.

PART III: Remarks

Documents cited anywhere in the Information Disclosure Statement are enclosed unless otherwise indicated. It is respectfully requested that:

1. The Examiner consider completely the cited information, along with any other information, in reaching a determination concerning the patentability of the present claims;
2. The enclosed form PTO-1449 (modified PTO/SB/08) be signed by the Examiner to evidence that the cited information has been fully considered by the United States Patent and Trademark Office during the examination of this application;
3. The citations for the information be printed on any patent which issues from this application.

By submitting this Information Disclosure Statement, the undersigned makes no representation that a search has been performed, of the extent of any search performed, or that more relevant information does not exist.

Application No.: 16/522,237
Conf. No.: 4736

- 3 -

Art Unit: 1649

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, in fact, prior art as defined by 35 U.S.C. § 102.

Notwithstanding any statements by the undersigned, the Examiner is urged to form his or her own conclusion regarding the relevance of the cited information.

An early and favorable action is hereby requested.

The Director is hereby authorized to charge any deficiency or credit any overpayment in the fees occasioned by the filing of this Information Disclosure Statement to our Deposit Account No. 23/2825 under Docket No. Q0052.70018US03 from which the undersigned is authorized to draw.

Respectfully submitted,

Electronic signature: /Michael J. Pomianek/
Michael J. Pomianek
Registration No.: 46,190
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Docket No.: Q0052.70018US03
Date: February 8, 2021

/Adam Weidner/

03/11/2021

FORM PTO-1449/A and B (modified PTO/SB/08) INFORMATION DISCLOSURE STATEMENT BY APPLICANT				APPLICATION NO.: 16/522,237		ATTY. DOCKET NO.: Q0052.70018US03	
				FILING DATE: July 25, 2019		CONFIRMATION NO.: 4736	
				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: A. M. Weidner	
Sheet	1	of	2				

U.S. PATENT DOCUMENTS

Examiner's Initials *	Cite No.	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication or Issue of Cited Document MM-DD-YYYY
		Number	Kind Code		
		10,640,814	B2	Duffy et al.	05-05-2020
		10,725,032	B2	Duffy et al.	07-28-2020
		2008-0039343	A1	Guire et al.	02-14-2008
		2008-0248962	A1	Kim et al.	10-09-2008
		2010-0124552	A1	Klein et al.	05-20-2010
		2011-0039278	A1	Pieribone	02-17-2011
		2012-0070379	A1	Black et al.	03-22-2012
		2014-0086836	A1	Burnham et al.	03-27-2014
		2015-0185232	A1	Keane et al.	07-02-2015
		2020-0271643	A1	Wilson et al.	08-27-2020
		2018-0037614	A1	Pollock et al.	02-02-2018
		2020-0393457	A1	Duffy et al.	12-17-2020

FOREIGN PATENT DOCUMENTS

Examiner's Initials *	Cite No.	Foreign Patent Document			Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Translation (Y/N)
		Office/Country	Number	Kind Code			
		EP	2 324 360	B1	Banyan Biomarkers, Inc.	01-31-2018	
		WO	91/06559	A1	E. I. Du Pont de Nemours and Company	05-16-1991	
		WO	2005/095262	A1	Nanyang Technological University	10-13-2005	
		WO	2013/103475	A2	Massachusetts Institute of Technology	07-11-2013	

OTHER ART - NON PATENT LITERATURE DOCUMENTS

Examiner's Initials *	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	Translation (Y/N)
		KAN et al., Isolation and detection of single molecules on paramagnetic beads using sequential fluid flows in microfabricated polymer array assemblies. Lab Chip. 2012 Mar 7;12(5):977-85. Epub 2011 Dec 16.	
		KOIKE et al., Oligemic hypoperfusion differentially affects tau and amyloid- β . Am J Pathol. 2010;177(1):300-310.	
EXAMINER:		DATE CONSIDERED:	

* EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

8931142.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /AMW/

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1002
000509

FORM PTO-1449/A and B (modified PTO/SB/08) INFORMATION DISCLOSURE STATEMENT BY APPLICANT				APPLICATION NO.: 16/522,237		ATTY. DOCKET NO.: Q0052.70018US03	
				FILING DATE: July 25, 2019		CONFIRMATION NO.: 4736	
				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: A. M. Weidner	
Sheet	2	of	2				

		WANG et al., Cerebrovascular hypoperfusion induces spatial memory impairment, synaptic changes, and amyloid- β oligomerization in rats. J Alzheimers Dis. 2010;21(3):813-822.				
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[NOTE - No copies of U.S. patents, published U.S. patent applications, or pending, unpublished patent applications stored in the USPTO's Image File Wrapper (IFW) system, are included. See 37 CFR § 1.98 and 1287OG163. Copies of all other patent(s), publication(s), unpublished, pending U.S. patent applications, or other information listed are provided as required by 37 CFR § 1.98 unless 1) such copies were provided in an IDS in an earlier application that complies with 37 CFR § 1.98, and 2) the earlier application is relied upon for an earlier filing date under 35 U.S.C. § 120.]

EXAMINER:	/Adam Weidner/	DATE CONSIDERED:	03/11/2021
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Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1002
000510

FORM PTO-1449/A and B (modified PTO/SB/08) INFORMATION DISCLOSURE STATEMENT BY APPLICANT				APPLICATION NO.: 16/522,237		ATTY. DOCKET NO.: Q0052.70018US03	
				FILING DATE: July 25, 2019		CONFIRMATION NO.: 4736	
				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
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U.S. PATENT DOCUMENTS

Examiner's Initials *	Cite No.	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication or Issue of Cited Document MM-DD-YYYY
		Number	Kind Code		
	***	3,712,986		Collings	01-23-1973
	***	4,200,110		Peterson et al.	04-29-1980
	***	4,232,119		Carlsson et al.	11-04-1980
	***	4,631,211		Houghten	12-23-1986
	***	4,780,421		Kameda et al.	10-25-1988
	***	4,883,642		Bisconte	11-28-1989
	***	4,907,037		Boisde et al.	03-06-1990
	***	4,924,870		Wlodarczyk et al.	05-15-1990
	***	4,962,037		Jett et al.	10-09-1990
	***	5,026,159		Allen et al.	06-25-1991
	***	5,028,535		Buechler et al.	07-02-1991
	***	5,089,391		Buechler et al.	02-18-1992
	***	5,091,300		Hurni et al.	02-25-1992
	***	5,108,961		Zhong et al.	04-28-1992
	***	5,152,816		Berkey	10-06-1992
	***	5,190,857		Allen et al.	03-02-1993
	***	5,196,306		Bobrow et al.	03-23-1993
	***	5,213,962		Van Nostrand et al.	05-05-1993
	***	5,234,814		Card et al.	08-10-1993
	***	5,244,636		Walt et al.	09-14-1993
	***	5,244,813		Walt et al.	09-14-1993
	***	5,250,264		Walt et al.	10-05-1993
	***	5,252,494		Walt	10-12-1993
	***	5,262,332		Selkoe et al.	11-16-1993
	***	5,270,165		Van Nostrand et al.	12-14-1993

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000511

FORM PTO-1449/A and B (modified PTO/SB/08) INFORMATION DISCLOSURE STATEMENT BY APPLICANT				APPLICATION NO.: 16/522,237		ATTY. DOCKET NO.: Q0052.70018US03	
				FILING DATE: July 25, 2019		CONFIRMATION NO.: 4736	
				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
Sheet	2	of	28				

	***	5,298,741		Walt et al.	03-29-1994
	***	5,315,375		Allen	05-24-1994
	***	5,320,814		Walt et al.	06-14-1994
	***	5,329,461		Allen et al.	07-12-1994
	***	5,374,395		Robinson et al.	12-20-1994
	***	5,405,783		Pirrung et al.	04-11-1995
	***	5,441,870		Seubert et al.	08-15-1995
	***	5,445,934		Fodor et al.	08-29-1995
	***	5,468,846		Ichikawa et al.	11-21-1995
	***	5,474,990		Olney	12-12-1995
	***	5,488,567		Allen et al.	01-30-1996
	***	5,492,812		Voorheis et al.	02-20-1996
	***	5,512,490		Walt et al.	04-30-1996
	***	5,532,138		Singh et al.	07-02-1996
	***	5,532,379		Fujimoto	07-02-1996
	***	5,545,531		Rava et al.	08-13-1996
	***	5,583,001		Bobrow et al.	12-10-1996
	***	5,593,846		Schenk et al.	01-14-1997
	***	5,620,850		Bamdad et al.	04-15-1997
	***	5,633,972		Walt et al.	05-27-1997
	***	5,690,894		Pinkel et al.	11-25-1997
	***	5,700,637		Southern	12-23-1997
	***	5,705,401		Masters et al.	01-06-1998
	***	5,731,158		Bobrow et al.	03-24-1998
	***	5,770,455		Cargill et al.	06-23-1998
	***	5,807,522		Brown et al.	09-15-1998
	***	5,814,524		Walt et al.	09-29-1998
	***	5,869,266		Wolozin et al.	02-09-1999
	***	5,885,529		Babson et al.	03-23-1999

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000512

FORM PTO-1449/A and B (modified PTO/SB/08) INFORMATION DISCLOSURE STATEMENT BY APPLICANT				APPLICATION NO.: 16/522,237		ATTY. DOCKET NO.: Q0052.70018US03	
				FILING DATE: July 25, 2019		CONFIRMATION NO.: 4736	
				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
Sheet	3	of	28				

	***	5,900,481		Lough et al.	05-04-1999
	***	5,976,817		Davies-Heerema et al.	11-02-1999
	***	5,981,180		Chandler et al.	11-09-1999
	***	5,981,208		Tamburini et al.	11-09-1999
	***	6,001,564		Bergeron et al.	12-14-1999
	***	6,007,690		Nelson et al.	12-28-1999
	***	6,013,445		Albrecht et al.	01-11-2000
	***	6,023,540		Walt et al.	02-08-2000
	***	6,133,436		Koster et al.	10-17-2000
	***	6,143,496		Brown et al.	11-07-2000
	***	6,156,270		Buechler	12-05-2000
	***	6,174,695	B1	Hammock et al.	01-16-2001
	***	6,210,910	B1	Walt et al.	04-03-2001
	***	6,266,459	B1	Walt et al.	07-24-2001
	***	6,285,807	B1	Walt et al.	09-04-2001
	***	6,327,410	B1	Walt et al.	12-04-2001
	***	6,329,139	B1	Nova et al.	12-11-2001
	***	6,368,874	B1	Gallop et al.	04-09-2002
	***	6,377,721	B1	Walt et al.	04-23-2002
	***	6,388,746	B1	Eriksson et al.	05-14-2002
	***	6,396,995	B1	Stuelpnagel et al.	05-28-2002
	***	6,406,845	B1	Walt et al.	06-18-2002
	***	6,432,630	B1	Blankenstein	08-13-2002
	***	6,482,593	B2	Walt et al.	11-19-2002
	***	6,495,335		Chojkier et al.	12-17-2002
	***	6,582,908	B2	Fodor et al.	06-24-2003
		6,599,331	B1	Chandler et al.	07-29-2003
	***	6,602,702	B1	McDevitt et al.	08-05-2003
	***	6,620,584	B1	Chee et al.	09-16-2003

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000513

FORM PTO-1449/A and B (modified PTO/SB/08) INFORMATION DISCLOSURE STATEMENT BY APPLICANT				APPLICATION NO.: 16/522,237		ATTY. DOCKET NO.: Q0052.70018US03	
				FILING DATE: July 25, 2019		CONFIRMATION NO.: 4736	
				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
Sheet	4	of	28				

	***	6,635,452	B1	Monforte et al.	10-21-2003
	***	6,667,159	B1	Walt et al.	12-23-2003
	***	6,670,137		Vanmechelen et al.	12-30-2003
	***	6,680,173		Vanmechelen et al.	01-20-2004
	***	6,713,309	B1	Anderson et al.	03-30-2004
	***	6,714,303	B2	Ivarsson	03-30-2004
	***	6,811,988		Chojkier et al.	11-02-2004
	***	6,821,449	B2	Caplen et al.	11-23-2004
	***	6,838,051	B2	Marquiss et al.	01-04-2005
	***	6,858,394	B1	Chee et al.	02-22-2005
	***	6,859,570	B2	Walt et al.	02-22-2005
	***	6,878,345	B1	Astle	04-12-2005
	***	6,929,924	B2	Bouanani et al.	08-16-2005
	***	6,942,968	B1	Dickinson et al.	09-13-2005
	***	6,943,034	B1	Winkler et al.	09-13-2005
	***	6,991,939	B2	Walt et al.	01-31-2006
	***	6,999,657	B2	Walt	02-14-2006
	***	7,056,746	B2	Seul et al.	06-06-2006
	***	7,060,431	B2	Chee et al.	06-13-2006
	***	7,107,092		Goldstein et al.	09-12-2006
	***	7,115,884	B1	Walt et al.	10-03-2006
	***	7,211,390	B2	Rothberg et al.	05-01-2007
	***	7,244,559	B2	Rothberg et al.	07-17-2007
	***	7,250,267	B2	Walt et al.	07-31-2007
	***	7,264,929	B2	Rothberg et al.	09-04-2007
	***	7,348,181	B2	Walt et al.	03-25-2008
	***	7,442,516		Ohno et al.	10-28-2008
	***	7,480,433	B2	Walt et al.	01-20-2009
	***	7,572,581	B2	Gelfand et al.	08-11-2009

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Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1002
000514

FORM PTO-1449/A and B (modified PTO/SB/08) INFORMATION DISCLOSURE STATEMENT BY APPLICANT				APPLICATION NO.: 16/522,237		ATTY. DOCKET NO.: Q0052.70018US03	
				FILING DATE: July 25, 2019		CONFIRMATION NO.: 4736	
				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
Sheet	5	of	28				

	***	7,651,841	B2	Song et al.	01-26-2010
	***	7,655,424	B2	Jackowski	02-02-2010
	***	7,749,700	B2	Baird et al.	07-06-2010
	***	7,759,062	B2	Allawi et al.	07-20-2010
	***	7,776,553	B2	Love et al.	08-17-2010
	***	7,838,250	B1	Goix et al.	11-23-2010
	***	8,222,047	B2	Duffy et al.	07-17-2012
	***	8,236,574	B2	Duffy et al.	08-07-2012
	***	8,415,171	B2	Rissin et al.	04-09-2013
	***	8,460,878	B2	Walt et al.	06-11-2013
	***	8,460,879	B2	Walt et al.	06-11-2013
	***	8,492,098	B2	Walt et al.	07-23-2013
	***	8,846,415	B2	Duffy et al.	09-30-2014
	***	9,110,025	B2	Rissin et al.	08-18-2015
	***	9,310,360	B2	Duffy et al.	04-12-2016
	***	9,395,359	B2	Walt et al.	07-19-2016
	***	9,482,622	B2	Sato et al.	11-01-2016
	***	9,482,662	B2	Duffy et al.	11-01-2016
	***	9,551,663	B2	Rissin et al.	01-24-2017
	***	9,678,068	B2	Duffy et al.	06-13-2017
	***	9,809,838		Walt et al.	11-07-2017
	***	9,846,155	B2	Rissin et al.	12-19-2017
	***	9,932,626	B2	Duffy et al.	04-03-2018
	***	9,952,237	B2	Fournier et al.	04-24-2018
		10,261,089	B2	Walt et al.	04-16-2019
		10,393,759	B2	Wilson et al.	08-27-2019
	***	2001-0018191	A1	Merken et al.	08-30-2001
	***	2002-0001857	A1	Vandermeeren et al.	01-03-2002
	***	2002-0009391	A1	Marquiss et al.	01-24-2002

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EX-1002
000515

FORM PTO-1449/A and B (modified PTO/SB/08) INFORMATION DISCLOSURE STATEMENT BY APPLICANT				APPLICATION NO.: 16/522,237		ATTY. DOCKET NO.: Q0052.70018US03	
				FILING DATE: July 25, 2019		CONFIRMATION NO.: 4736	
				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
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	***	2002-0019016	A1	Vanmechelen et al.	02-14-2002
	***	2002-0086009	A1	Ishiguro et al.	07-04-2002
	***	2002-0090650	A1	Empedocles et al.	07-11-2002
	***	2002-0122612	A1	Walt et al.	09-05-2002
	***	2002-0137112	A1	Chojkier et al.	09-26-2002
	***	2002-0157122	A1	Wong et al.	10-24-2002
	***	2002-0188106	A1	Mandelkow et al.	12-12-2002
	***	2003-0027126	A1	Walt et al.	02-06-2003
	***	2003-0091475	A1	Yu et al.	05-15-2003
	***	2003-0096227	A1	Shinitzky et al.	05-22-2003
	***	2003-0096243	A1	Busa	05-22-2003
	***	2003-0104361	A1	Weininger et al.	06-05-2003
	***	2003-0104488	A1	Chojkier et al.	06-05-2003
	***	2003-0138972	A1	Vandermeeren et al.	07-24-2003
	***	2003-0143580	A1	Straus et al.	07-31-2003
	***	2003-0143760	A1	Vandermeeren et al.	07-31-2003
	***	2003-0165575	A1	Iqbal. et al.	09-04-2003
	***	2003-0194742	A1	Vanmechelen et al.	10-16-2003
	***	2003-0198573	A1	Forood et al.	10-23-2003
	***	2004-0013647	A1	Solomon et al.	01-22-2004
	***	2004-0014142	A1	Vanmechelen et al.	01-22-2004
	***	2004-0018561	A1	Decrescenzo et al.	01-29-2004
	***	2004-0038426	A1	Manalis	02-26-2004
	***	2004-0038430	A1	Vandermeeren et al.	02-26-2004
	***	2004-0043502	A1	Song et al.	03-04-2004
	***	2004-0053322	A1	McDevitt et al.	03-18-2004
	***	2004-0071599	A1	Rusch et al.	04-15-2004
	***	2004-0072278	A1	Chou et al.	04-15-2004
	***	2004-0086426	A1	Vann et al.	05-06-2004

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000516

FORM PTO-1449/A and B (modified PTO/SB/08) INFORMATION DISCLOSURE STATEMENT BY APPLICANT				APPLICATION NO.: 16/522,237		ATTY. DOCKET NO.: Q0052.70018US03	
				FILING DATE: July 25, 2019		CONFIRMATION NO.: 4736	
				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
Sheet	7	of	28				

	***	2004-0101918	A1	Cauci	05-27-2004
	***	2004-0132680	A1	Wong et al.	07-08-2004
	***	2004-0142386	A1	Rigler et al.	07-22-2004
	***	2004-0166536	A1	Kerkman et al.	08-26-2004
	***	2004-0219509	A1	Valkirs et al.	11-04-2004
	***	2004-0248325	A1	Bukusoglu	12-09-2004
	***	2004-0253624	A1	Smith et al.	12-16-2004
	***	2004-0253643	A1	Seubert et al.	12-16-2004
	***	2004-0259237	A1	Kellogg et al.	12-23-2004
	***	2005-0042221	A1	Chojkier et al.	02-24-2005
	***	2005-0053996	A1	Tong	03-10-2005
	***	2005-0069968	A1	Seubert et al.	03-31-2005
	***	2005-0112634	A1	Woudenberg et al.	05-26-2005
	***	2005-0112655	A1	Banerjee et al.	05-26-2005
	***	2005-0124016	A1	LaDu et al.	06-09-2005
	***	2005-0129581	A1	McBride et al.	06-16-2005
	***	2005-0130173	A1	Leamon et al.	06-16-2005
	***	2005-0130188	A1	Walt et al.	06-16-2005
	***	2005-0131650	A1	Andersson et al.	06-16-2005
	***	2005-0164289	A1	Quate et al.	07-28-2005
	***	2005-0169925	A1	Bardroff et al.	08-04-2005
	***	2005-0175626	A1	Delacourte et al.	08-11-2005
	***	2005-0181460	A1	Ohno et al.	08-15-2005
	***	2005-0221281	A1	Ho	10-06-2005
	***	2005-0226780	A1	Sandell et al.	10-13-2005
	***	2005-0244308	A1	Tanaami et al.	11-03-2005
	***	2005-0244890	A1	Davies et al.	11-03-2005
	***	2005-0266433	A1	Kapur et al.	12-01-2005
	***	2006-0006067	A1	Unger	01-12-2006

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FORM PTO-1449/A and B (modified PTO/SB/08) INFORMATION DISCLOSURE STATEMENT BY APPLICANT				APPLICATION NO.: 16/522,237		ATTY. DOCKET NO.: Q0052.70018US03	
				FILING DATE: July 25, 2019		CONFIRMATION NO.: 4736	
				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
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	***	2006-0013543	A1	Walt et al.	01-19-2006
	***	2006-0024759	A1	Holtzman et al.	02-02-2006
	***	2006-0040297	A1	Leamon et al.	02-23-2006
	***	2006-0068409	A1	Phan et al.	03-30-2006
	***	2006-0078998	A1	Puskas et al.	04-13-2006
	***	2006-0084183	A1	Henricksen	04-20-2006
	***	2006-0139635	A1	Kersey et al.	06-29-2006
	***	2006-0205024	A1	Rogers et al.	09-14-2006
	***	2007-0040095	A1	Walt et al.	02-22-2007
	***	2007-0059754	A1	Kordunsky et al.	03-15-2007
	***	2007-0074972	A1	Nassef et al.	04-05-2007
	***	2007-0116607	A1	Wang et al.	05-24-2007
	***	2007-0154480	A1	Schenk et al.	07-05-2007
	***	2007-0259381	A1	Rissin et al.	11-08-2007
	***	2007-0259385	A1	Rissin et al.	11-08-2007
	***	2007-0259448	A1	Rissin et al.	11-08-2007
	***	2008-0032324	A1	Walt et al.	02-07-2008
	***	2008-0038761	A1	Beernink et al.	02-14-2008
	***	2008-0064113	A1	Goix et al.	03-13-2008
	***	2008-0118924	A1	Buechler	05-22-2008
	***	2008-0131422	A1	Sugimura, et al.	06-05-2008
	***	2008-0131907	A1	Wang et al.	06-05-2008
	***	2008-0199879	A1	Takayama et al.	09-21-2008
	***	2008-0234311	A1	Li et al.	09-25-2008
		2008-0254482	A1	Mattoon et al.	10-16-2008
	***	2008-0269069	A1	Bacher et al.	10-30-2008
	***	2008-0299111	A1	Delacourte et al.	12-04-2008
	***	2009-0022825	A1	Kerkman et al.	01-22-2009
	***	2009-0036324	A1	Fan et al.	02-05-2009

EXAMINER:	DATE CONSIDERED:
-----------	------------------

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				FILING DATE: July 25, 2019		CONFIRMATION NO.: 4736	
				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
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	***	2009-0087860	A1	Todd et al.	04-02-2009
	***	2009-0142755	A1	Albitar	06-04-2009
	***	2009-0149341	A1	Walt et al.	06-11-2009
	***	2009-0156425	A1	Walt et al.	06-18-2009
	***	2009-0170728	A1	Walt et al.	07-02-2009
	***	2009-0239308	A1	Dube et al.	09-24-2009
	***	2009-0254180	A1	Pazanowski et al.	10-08-2009
	***	2009-0289834	A1	Devensky	11-26-2009
	***	2009-0307772	A1	Markham et al.	12-10-2009
	***	2010-0075355	A1	Duffy et al.	03-25-2010
	***	2010-0075407	A1	Duffy et al.	03-25-2010
	***	2010-0075439	A1	Duffy et al.	03-25-2010
	***	2010-0075862	A1	Duffy et al.	03-25-2010
	***	2010-0136593	A1	Jackowski	06-03-2010
	***	2010-0140289	A1	Knobel et al.	06-10-2010
	***	2010-0189338	A1	Lin et al.	07-29-2010
	***	2010-0192573	A1	Hamilton et al.	08-05-2010
	***	2010-0204335	A1	Beddingfield et al.	08-12-2010
	***	2010-0225913	A1	Trainer	09-09-2010
	***	2010-0227379	A1	Wo et al.	09-09-2010
	***	2010-0267582	A1	Baird et al.	10-21-2010
	***	2010-0329929		Goix et al.	12-30-2010
	***	2011-0037463	A1	Bertacco et al.	02-17-2011
	***	2011-0183330	A1	Lo et al.	07-28-2011
	***	2011-0195852	A1	Walt et al.	08-11-2011
	***	2011-0212462	A1	Duffy et al.	09-01-2011
	***	2011-0212537	A1	Rissin et al.	09-01-2011
	***	2011-0212848	A1	Duffy et al.	09-01-2011
	***	2011-0245097	A1	Rissin et al.	10-06-2011

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				FILING DATE: July 25, 2019		CONFIRMATION NO.: 4736	
				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
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	***	2012-0183967	A1	Dressman et al.	07-19-2012
	***	2012-0196774	A1	Fournier et al.	08-02-2012
	***	2012-0214160	A1	Deng et al.	08-23-2012
	***	2012-0277114	A1	Duffy et al.	11-01-2012
	***	2012-0289428	A1	Duffy et al.	11-15-2012
	***	2013-0165342	A1	Rissin et al.	06-27-2013
	***	2013-0345078	A1	Walt et al.	12-26-2013
	***	2014-0094386	A1	Wilson et al.	04-03-2014
	***	2014-0227720	A1	Wilson et al.	08-14-2014
	***	2014-0302532	A1	Wilson et al.	10-09-2014
	***	2015-0233905	A1	Walt et al.	08-20-2015
	***	2015-0353997	A1	Duffy et al.	12-10-2015
	***	2015-0355182	A1	Rissin et al.	12-10-2015
	***	2016-0123969	A1	Rissin et al.	05-05-2016
	***	2016-0258959		Wilson et al.	09-08-2016
	***	2017-0038390	A1	Walt et al.	02-07-2017
	***	2017-0159104	A1	Walt et al.	06-08-2017
		2017-0160292	A1	Wilson et al.	06-08-2017
	***	2018-0003703	A1	Duffy et al.	01-04-2018
	***	2018-0017552	A1	Duffy et al.	01-18-2018
	***	2018-0224451	A1	Rissin et al.	08-09-2018
	***	2018-0306830	A1	Fournier et al.	10-25-2018
		2018-0363038	A1	Duffy et al.	12-20-2018
		2019-0293655	A1	Walt et al.	09-26-2019
		2019-0302109	A1	Duffy et al.	10-03-2019

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				FILING DATE: July 25, 2019		CONFIRMATION NO.: 4736	
				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
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FOREIGN PATENT DOCUMENTS

Examiner's Initials *	Cite No.	Foreign Patent Document			Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Translation (Y/N)
		Office/Country	Number	Kind Code			
	**	AU	199956253	B2	Febit Ferrarius Biotechnology GmbH	03-21-2000	
	**	CN	1635146	A	Hunan University	07-06-2005	Y - Abstract Only
	**	CN	1950520	A	Siemens AG	04-18-2007	Y - Abstract Only
	***	CN	101351564	A	Digene Corp	01-21-2009	Y - Abstract Only
	***	CN	101529227	A	Univ Singapore	09-09-2009	Y - Abstract Only
	***	CN	101541974	A	Glaxosmithkline Biolog SA	09-23-2009	Y - Abstract Only
	**	DE	19540098	A1	Inst Chemo Biosensorik	04-30-1997	Y - Abstract Only
	**	EP	0 805 215	A2	Vysis, Inc	11-05-1997	
	**	EP	1 160 256	A2	Bayer AG	12-05-2001	
	**	EP	1 180 679	A1	Evotec Ag	02-20-2002	
	**	EP	1 259 810	A2	Trutnau, Hans-heinrich	11-27-2002	Y - see abstract of counterpart WO 01/57520, cited herein
	**	EP	1 298 436	A2	Elan Pharm Inc	04-02-2003	
	**	EP	1 721 657	A1	Sony Deutschland GmbH	11-15-2006	
	**	EP	1 978 035	A1	Hans Knoell Inst Leibniz	10-08-2008	
	**	EP	2 267 451	A2	Life Technologies Corporation	12-29-2010	
	**	JP	2001-269196	A	Hamamatsu Photonics Kk	10-02-2001	Y
	**	JP	2002-506200	A	Tufts College et al.	02-26-2002	See counterpart WO 99/45357
	**	JP	2002-525587	A	Febit Ferrarius Biotechnology GmbH	08-13-2002	See counterpart AU 199956253
	**	JP	2002-526743	A	DDX, Inc.	08-20-2002	See counterpart WO 99/58948
	**	JP	2004-354164	A	Olympus Corp.	12-16-2004	Y
	**	JP	2005-518553	A	Genomic Profiling Systems, Inc.	06-23-2005	See counterpart WO 03/073817
	**	JP	2006-511792	A	Smith et al.	04-06-2006	See counterpart US 2004-253624
	**	WO	88/05533	A1	Kelsius, Inc	07-28-1988	

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-----------	------------------

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				FIRST NAMED INVENTOR: David Wilson			
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	**	WO	93/06121	A1	Affymax Tech Nv	04-01-1993	
	**	WO	93/24517	A2	Furka et al.	12-09-1993	
	**	WO	95/19178	A1	Research Foundation for Mental Hygiene Inc.	07-20-1995	
	**	WO	95/25116	A1	California Inst Of Techn	09-21-1995	
	**	WO	95/32425	A1	Smithkline Beecham Corp	11-30-1995	
	**	WO	95/35506	A2	Kensington Laboratories, Inc	12-28-1995	
	**	WO	96/04309	A1	Innogenetics NV	02-15-1996	
	**	WO	97/27326	A1	Univ California	07-31-1997	
	**	WO	98/50782	A2	Tufts College	11-12-1998	
	**	WO	99/45357	A2	Tufts College et al.	09-10-1999	
	**	WO	99/58948	A2	DDX, Inc.	11-18-1999	
	**	WO	00/04372	A1	The Board Of Regents, The University Of Texas System	01-27-2000	
	**	WO	00/14546	A1	Innogenetics, N.V.	03-16-2000	
	**	WO	00/47996	A2	Illumina, Inc	08-17-2000	
	**	WO	01/57520	A2	Trutnau, Hans-heinrich	08-09-2001	Y Abstract Only
	**	WO	01/90757	A1	University Court of the University of Dundee	11-29-2001	
	**	WO	02/46222	A2	Mario Chojkier	06-13-2002	
	**	WO	02/47466	A2	The Johns Hopkins University School of Medicine	06-20-2002	
	**	WO	03/054142	A2	454 Corp	07-03-2003	
	**	WO	03/073817	A1	Genomic Profiling Systems, Inc.	09-12-2003	
	**	WO	2004/043226	A2	Molecular Geriatrics Corporation	05-27-2004	
	**	WO	2004/065000	A1	Illumina Inc	08-05-2004	
	**	WO	2004/083443	A1	Caliper Techn Corp	09-30-2004	
	**	WO	2005/011599	A2	Northwestern University	02-10-2005	
	**	WO	2005/019419	A2	Singulex, Inc	03-03-2005	
	**	WO	2005/023414	A1	Illumina, Inc	03-17-2005	
	**	WO	2005/033283	A2	Singulex, Inc	04-14-2005	

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	**	WO	2005/047484	A2	Ciphergen Biosystems Inc.	05-26-2005	
	**	WO	2005/054431	A2	454 Corp	06-16-2005	
	**	WO	2005/075507	A1	Affibody AB	08-18-2005	
	**	WO	2005/123775	A1	Bioarctic Neuroscience AB	12-29-2005	
	**	WO	2006/007726	A1	Umedik Inc. et al.	01-26-2006	
	**	WO	2006/055739	A2	Sack, Robert	05-26-2006	
	**	WO	2006/078289	A2	Nanosphere, Inc	07-27-2006	
	**	WO	2006/102297	A1	Applera Corp et al.	09-28-2006	
	**	WO	2006/103116	A1	Biotherpix Molecular Medicines S.L.U.	10-05-2006	
	**	WO	2006/108180	A2	454 Life Sciences Corp	10-12-2006	
	**	WO	2007/016357	A1	Regnets of the University of Minnesota	02-08-2007	
	**	WO	2007/044091	A2	Fluidigm Corp	04-19-2007	
	**	WO	2007/044974	A2	Univ New York State Res Found	04-19-2007	
	**	WO	2007/050359	A2	Merck & Co. Inc.	05-03-2007	
	**	WO	2007/064972	A2	Abbott Laboratories	06-07-2007	
	**	WO	2007/068429	A1	F. Hoffmann-La Roche AG	06-21-2007	
	**	WO	2007/081385	A2	Raindance Technologies, Inc	07-19-2007	
	**	WO	2007/081386	A2	Raindance Technologies, Inc	07-19-2007	
	**	WO	2007/081387	A1	Raindance Technologies, Inc	07-19-2007	
	**	WO	2007/084192	A2	Univ California	07-26-2007	
	**	WO	2007/082750	A1	Univ Friedrich Alexander	07-26-2007	
	**	WO	2007/090126	A2	Invitrogen Corporation	08-09-2007	
	**	WO	2007/098148	A2	Univ Tufts	08-30-2007	
	**	WO	2007/106762	A2	Washington University in St. Louis	09-20-2007	
	**	WO	2007/114947	A2	Singulex, Inc	10-11-2007	
	**	WO	2007/136614	A2	Merck & Co. Inc.	11-29-2007	
	**	WO	2008/027017	A1	Singapore Health Services PTE LTD	03-06-2008	
	**	WO	2008/034016	A2	FoldRX Pharmaceuticals Inc.	03-20-2008	
	**	WO	2008/048371	A2	Singulex, Inc	04-24-2008	
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	**	WO	2008/060364	A2	AC Immune SA	05-22-2008	
	**	WO	2008/067464	A2	Abbott Laboratories	06-05-2008	
	**	WO	2008/081008	A1	University of Zurich	07-10-2008	
	**	WO	2008/150946	A1	Abbott Laboratories	12-11-2008	
	**	WO	2008/156622	A1	AC Immune SA	12-24-2008	
	**	WO	2009/004494	A2	Institut Pasteur	01-08-2009	
	**	WO	2009/029073	A1	Tufts College	03-05-2009	
	**	WO	2009/156747	A3	The Nottingham Trent University	12-30-2009	
	**	WO	2010/039180	A2	Quanterix Corp	04-08-2010	
	**	WO	2011/032155	A2	Banyan Biomarkers Inc.	03-17-2011	
	**	WO	2011/109372	A1	Quanterix Corp.	09-09-2011	
	***	WO	2011/109364	A2	Quanterix Corp	09-09-2011	
	***	WO	2016/115256	A1	Quanterix Corp	07-21-2016	
	***	WO	2016/130923	A1	Quanterix Corp	08-18-2016	
		WO	2018/222585	A2	Quanterix Corp	12-06-2018	
		WO	2019/060607	A1	Quanterix Corp	03-28-2019	

OTHER ART - NON PATENT LITERATURE DOCUMENTS

Examiner's Initials #	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	Translation (Y/N)
	**	International Search Report and Written Opinion mailed November 29, 2012 for Application No. PCT/US2012/033338 (Q0052.70017WO00).	
	**	International Preliminary Report on Patentability mailed October 24, 2013 for Application No. PCT/US2012/033338 (Q0052.70017WO00).	
		Office Communication for US App. No. 14/111,331 mailed December 12, 2019 and claims pending (Q0052.70017US01).	
	**	International Search Report and Written Opinion mailed October 29, 2012 for Application No. PCT/US2012/033343 (Q0052.70018WO00).	
	**	International Preliminary Report on Patentability mailed October 24, 2013 for Application No. PCT/US2012/033343 (Q0052.70018WO00).	
	**	[NO AUTHOR LISTED], bioMérieux and Quanterix Sign Strategic Partnership in Ultrasensitive and Multiplex Immunoassays. Quanterix Press Release. 2012 Nov 15. 2 pages.	

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-----------	------------------

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				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
Sheet	15	of	28				

**	[NO AUTHOR LISTED], Does Brain Hypoxia Help Kick Off Alzheimer's Pathology? Alzheimer Research Forum. 2011 Dec 16. http://www.alzforum.org/new/detailprint.asp?id=3002 [last accessed 30 January 2012]. 4 pages.	
**	[NO AUTHOR LISTED], Novel test following prostate surgery could detect cancer recurrence earlier. AACR Press Release. 2010 Sep 29. Last accessed at http://www.aacr.org/home/public-media/aacr-press-releases.aspx?d=2072 on 1/31/2012. 2 pages.	
**	[NO AUTHOR LISTED], Pittcon Announces 2010 Technical Program: Webcast of Selected Symposia. Press Release. 2009 Oct 15. http://archive.constantcontact.com/fs033/1102032821298/archive/1102745632000.html [last accessed 31 January 2012]. 2 pages.	
**	[NO AUTHOR LISTED], Quanterix and STRATEC Announce Strategic Partnership. Quanterix Press Release. 2011 Aug 16. 2 pages.	
**	[NO AUTHOR LISTED], Quanterix Announces Commercial Availability of its Simoa Single Molecule Array Technology. Quanterix Press Release. 2013 Jul 30. 2 pages.	
**	[NO AUTHOR LISTED], Quanterix corporation awarded \$185,000 grant from the National Cancer Institute. Quanterix Press Release. 2008 Sep 30. Last accessed at http://www.quanterix.com/news/pressReleases/SBIR1Grant.html on 1/31/2012. 1 page.	
**	[NO AUTHOR LISTED], Quanterix corporation raises \$15 million in series A financing. Quanterix Press Release. 2008 Aug 25. Last accessed at http://www.quanterix.com/news/pressReleases/seriesAFunding.html on 1/31/2012. 2 pages.	
**	[NO AUTHOR LISTED], Quanterix Digital ELISA Measures Low Abundance Biomarkers of Inflammation in Crohn's Disease. Quanterix Press Release. 2011 August 19. Last accessed at http://www.quanterix.com/events-news/press-releases/item/142-quanterix-digital-elisa-measures-low-abundance-biomarkers-of-inflammation-in-crohn's-disease on 9/20/12.	
**	[NO AUTHOR LISTED], Quanterix Discovers Link Between Heart Attack-induced Hypoxia and Suspected Alzheimer's Disease Pathway. Quanterix Press Release. 2011 12 April. Last accessed at http://www.quanterix.com/events-news/press-releases/item/146-quanterix-discovers-link-between-heart-attack-induced-hypoxia-and-suspected-alzheimer's-disease-pathway on 9/20/2012.	
**	[NO AUTHOR LISTED], Quanterix Launches Multiplexed Single Molecule Immunoassay Technology to Improve Diagnosis and Potential Treatment of Complex Diseases. Quanterix Press Release. 2013 Sep 17. 2 pages.	
**	[NO AUTHOR LISTED], Quanterix to Present Poster Session on Blood-based Brain Biomarker Measurements of Sports Related Brain Injury at Neuroscience. Quanterix Press Release. 2013 Nov 4. 1 page.	
**	[NO AUTHOR LISTED], Quanterix's Simoa technology to detect blood biomarker for concussion in hockey players. Quanterix Press Release. 2014 Mar 14. 1 page.	

EXAMINER:	DATE CONSIDERED:
-----------	------------------

* EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609: Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

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7510714.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /AMW/

FORM PTO-1449/A and B (modified PTO/SB/08) INFORMATION DISCLOSURE STATEMENT BY APPLICANT				APPLICATION NO.: 16/522,237		ATTY. DOCKET NO.: Q0052.70018US03	
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				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
Sheet	16	of	28				

**	[NO AUTHOR LISTED], Quanterix's Ultrasensitive Simoa™ Technology Demonstrates Equivalence with NAT and 3,000x Improvement in Sensitivity over Conventional Immunoassays for HIV Detection. Quanterix Press Release. 2012 Oct 11. 1 page.	
**	[NO AUTHOR LISTED], Quanterix's Ultrasensitive Simoa™ Technology Forges New Ground with Direct Detection of Genomic DNA in Human Blood and River Water. Quanterix Press Release. 2013 Jan 22. 2 pages.	
**	[NO AUTHOR LISTED], Scientific Principle of Simoa™ (Single Molecule Array) Technology. Whitepaper 1.0. 2013 Jul 19. 2 pages.	
**	[NO AUTHOR LISTED], Single molecule arrays for digital detection in complex samples. Quanterix Corporation. IQT Technology Focus Day. March 25, 2010. PowerPoint presentation. 30 pages.	
**	[NO AUTHOR LISTED], Ultrasensitive Tau Assay Enables Quantification of Neuronal Biomarker in Blood for the First Time. Whitepaper 3.0. Quanterix. 2013 Oct 8. 4 pages.	
**	ADAMS et al., Encoded fiber-optic microsphere arrays for probing protein-carbohydrate interactions. Angewandte Chemie. 2003; 115:5475-5478.	
**	AGRAWAL et al., Nanometer-scale mapping and single-molecule detection with color-coded nanoparticle probes. Proc Natl Acad Sci U S A. 2008 Mar 4;105(9):3298-303. Epub 2008 Feb 27.	
**	AGRAWAL et al., Single-bead immunoassays using magnetic microparticles and spectral-shifting quantum dots. J Agric Food Chem. 2007 May 16; 55(10):3778-82. Epub 2007 Apr 25.	
**	AHN et al., Detection of Salmonella spp. Using microsphere-based, fiber-optic DNA microarrays. Anal Chem. 2005 Aug 1; 77(15):5041-7.	
**	AHN et al., Fiber-optic microarray for simultaneous detection of multiple harmful algal bloom species. Appl Environ Microbiol. 2006 Sep; 72(9):5742-9.	
**	ALBERT et al., Automatic decoding of sensor types within randomly ordered, high-density optical sensor arrays. Anal Bioanal Chem. 2002 Apr; 373(8):792-802. Epub 2002 Jul 27.	
**	ALBERT et al., Cross-reactive chemical sensor arrays. Chem Rev. 2000 Jul 12; 100(7):2595-626.	
**	ALBERT et al., Information coding in artificial olfaction ultrasensory arrays. Anal Chem. 2003 Aug 15; 75(16):4161-7.	
**	ALBERT et al., Optical multibead arrays for simple and complex odor discrimination. Anal Chem. 2001 Jun 1; 73(11):2501-8.	
**	ANGENENDT et al., Subnanoliter enzymatic assays on microarrays. Proteomics. 2005 Feb;5(2):420-5	
**	ARNAUD, Observing single enzymes at work. Chemical & Engineering News. October 2007; 85(44): 8.	
**	BEER et al., On-chip, real-time, single-copy polymerase chain reaction in picoliter droplets. Anal Chem. 2007 Nov 15;79(22):8471-5. Epub 2007 Oct 11. Abstract only.	

EXAMINER:	DATE CONSIDERED:
-----------	------------------

* EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

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7510714.1

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				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
Sheet	17	of	28				

**	BENCIC-NAGALE et al., Extending the longevity of fluorescence-based sensor arrays using adaptive exposure. Anal Chem. 2005 Oct 1; 77(19):6155-62.	
**	BHAT et al., Single molecule detection in nanofluidic digital array enables accurate measurement of DNA copy number. Anal Bioanal Chem. 2009 May;394(2):457-67. Epub 2009 Mar 15.	
**	BIRAN et al., Optical imaging fiber-based live bacterial cell array biosensor. Anal Biochem. 2003 Apr 1; 315(1):106-13.	
**	BIRAN et al., Optical imaging fiber-based single live cell arrays: a high-density cell assay platform. Anal Chem. 2002 Jul 1; 74(13):3046-54.	
**	BLAKE et al., Phenotypic consequences of promoter-mediated transcriptional noise. Mol Cell. 2006 Dec 28; 24(6):853-65.	
**	BLICHARZ et al., Detection of inflammatory cytokines using a fiber optic microsphere immunoassay array. Proc. SPIE. 2006; 6380, 638010-1-638010-6.	
**	BLICHARZ et al., Fiber-optic microsphere-based antibody array for the analysis of inflammatory cytokines in saliva. Anal. Chem. 2009;81(6):2106-14.	
**	BLICHARZ et al., Use of colorimetric test strips for monitoring the effect of hemodialysis on salivary nitrite and uric acid in patients with end-stage renal disease: a proof of principle. Clin Chem. 2008 Sep; 54(9):1473-80. Epub 2008 Aug 1.	
**	BOURZAC, Next-generation diagnostics: a startup can detect tiny traces of cancer markers in blood samples. Technol Rev. 2008 May 13. Last accessed at http://www.technologyreview.com/Biztech/20760/?a=f on 2/2/2012. 2 pages.	
**	BOWDEN et al., Development of a microfluidic platform with an optical imaging microarray capable of attomolar target DNA detection. Anal Chem. 2005 Sep 1; 77(17):5583-8.	
**	BOYDEN, The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. J Exp Med. 1962 Mar 1;115:453-66.	
**	BREHM-STECHER et al., Single-cell microbiology: tools, technologies, and applications. Microbiol Mol Biol Rev. 2004 Sep; 68(3):538-59.	
**	BROGAN et al., Optical fiber-based sensors: application to chemical biology. Curr Opin Chem Biol. 2005 Oct; 9(5):494-500.	
**	BRONK et al., Combined imaging and chemical sensing using a single optical imaging fiber. Anal Chem. 1995 Sep 1; 67(17):2750-7.	
**	BRONK et al., Fabrication of patterned sensor arrays with aryl azides on a polymer-coated imaging optical fiber bundle. Anal Chem. 1994 Oct 15; 66(20):3519-20.	
**	BULUT et al., Tau protein as a serum marker of brain damage in mild traumatic brain injury: preliminary results. Adv Ther. 2006 Jan-Feb;23(1):12-22.	
**	BURTON et al., A microfluidic chip-compatible bioassay based on single-molecule detection with high sensitivity and multiplexing. Lab Chip. 2010 Apr 7; 10(7):843-51. Epub 2010 Jan 14.	

EXAMINER:	DATE CONSIDERED:
-----------	------------------

* EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609: Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

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				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
Sheet	18	of	28				

**	CAMPION, Colored and fluorescent solid supports. Innovation and Perspectives in Solid Phase Synthesis. Ed. R. Epton, Mayflower Worldwide Limited, Birmingham. Ch. 77. 1994:469-472.	
**	CHANG et al., Digital ELISA of HIV P24 capsid protein with sensitivity of nucleic acid amplification tests. 2012 AACC Meeting. Los Angeles, CA. Abstract and Poster. 2012. 2 pages.	
**	CHANG et al., Prototype digital immunoassay for troponin I with sub-femtomolar sensitivity. 2013 AACC Meeting. Houston, TX. Abstract and Poster. 2013. 2 pages.	
**	CHANG et al., Simple diffusion-constrained immunoassay for p24 protein with the sensitivity of nucleic acid amplification for detecting acute HIV infection. J Virol Methods. 2013 Mar;188(1-2):153-60. doi: 10.1016/j.jviromet.2012.08.017. Epub 2012 Oct 2.	
**	CHANG et al., Single molecule enzyme-linked immunosorbent assays: theoretical considerations. J Immunol Methods. 2012 Apr 30;378(1-2):102-15. doi: 10.1016/j.jim.2012.02.011. Epub 2013 Apr 30. 28 pages.	
**	CHEN et al., Microfabricated arrays of cylindrical wells facilitate single-molecule enzymology of alpha-chymotrypsin. Biotechnol Prog. 2009 Jul-Aug; 25(4):929-37.	
**	CHIN et al., Editor's Choice: Distinctive individualism. Science. 2008 Apr 4;320:21.	
**	CHON et al., Characterization of single-cell migration using a computer-aided fluorescence time-lapse videomicroscopy system. Anal Biochem. 1997 Oct 15;252(2):246-54.	
**	DEUTSCH et al., Apparatus for high-precision repetitive sequential optical measurement of living cells. Cytometry. 1994 Jul 1; 16(3):214-26.	
**	DICESARE et al., Individual cell migration analysis using fiber-optic bundles. Anal Bioanal Chem. 2005 May; 382(1):37-43. Epub 2005 Apr 1.	
**	DICKINSON et al., A chemical-detecting system based on a cross-reactive optical sensor array. Nature. 1996 Aug 22; 382(6593):697-700.	
**	DICKINSON et al., Convergent, self-encoded bead sensor arrays in the design of an artificial. Anal Chem. 1999 Jun 1; 71(11):2192-8.	
**	DICKINSON et al., Current trends in 'artificial-nose' technology. Trends Biotechnol. 1998 Jun; 16(6):250-8.	
**	DOLINAK et al., Global hypoxia per se is an unusual cause of axonal injury. Acta Neuropathol. 2000;100:553-60.	
**	DUFFY et al., Detection of prostate specific antigen (PSA) in the serum of radical prostatectomy patients at femtogram per milliliter levels using digital ELISA (AccuPSATM) based on single molecule arrays (SiMoA). AACC Meeting Poster. 2010. 1 page.	
**	DUFFY, Immunoassays with Broad Dynamic Ranges based on Combining Digital and Digitally Enhanced Analog Detection of Enzyme Labels. Oak Ridge Conference. Presentation. 2011 Apr 15. 16 pages.	
**	DUFFY, Single Molecule Arrays (Simoa) for Ultrasensitive Protein Detection in Companion Diagnostics. Next Generation DX Summit. August 22, 2012. PowerPoint presentation. 36 slides.	

EXAMINER:	DATE CONSIDERED:
-----------	------------------

* EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609: Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

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				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
Sheet	19	of	28				

**	DUFFY, Ultra-sensitive protein detection using single molecule arrays (Simoa): the potential for detecting single molecules of botulinum toxin. The Botulinum J. 2012;2(2):164-7.	
**	EGNER et al., Tagging in combinatorial chemistry: the use of coloured and fluorescent beads. Chem Commun. 1997; 735-736.	
**	EID et al., Real-time DNA sequencing from single polymerase molecules. Science. 2009 Jan 2; 323(5910):133-8. Epub 2008 Nov 20.	
**	EKINS et al., Single-molecule ELISA. Clin Chem. 2011 Mar;57(3):372-5. Epub 2010 Oct 13. Papers in press. 2010 Oct 13. Pages 1-3.	
**	ENGLISH et al., Ever-fluctuating single enzyme molecules: Michaelis-Menten equation revisited. Nat Chem Biol. 2006 Feb; 2(2):87-94. Epub 2005 Dec 25.	
**	EPSTEIN et al., Combinatorial decoding: an approach for universal DNA array fabrication. J Am Chem Soc. 2003 Nov 12; 125(45):13753-9.	
**	EPSTEIN et al., Fluorescence-based nucleic acid detection and microarrays. Analytica Chimica Acta. 2002; 469:3-36.	
**	EPSTEIN et al., High-density fiber-optic genosensor microsphere array capable of zeptomole detection limits. Anal Chem. 2002 Apr 15; 74(8):1836-40.	
**	EPSTEIN et al., High-density, microsphere-based fiber optic DNA microarrays. Biosens Bioelectron. 2003 May; 18(5-6):541-6.	
**	EPSTEIN, et al., Fluorescence-based fibre optic arrays: a universal platform for sensing. Chem Soc Rev. 2003 Jul; 32(4):203-14.	
**	FERGUSON et al., A fiber-optic DNA biosensor microarray for the analysis of gene expression. Nat Biotechnol. 1996 Dec; 14(13):1681-4.	
**	FERGUSON et al., High-density fiber-optic DNA random microsphere array. Anal Chem. 2000 Nov 15; 72(22):5618-24.	
**	FERGUSON et al., Simultaneous monitoring of pH, CO ₂ and O ₂ using an optical imaging fiber. Analytica Chimica Acta. 1997; 340(1-3):123-131.	
**	FISTER et al., Counting single chromophore molecules for ultrasensitive analysis and separations on microchip devices. Analytical Chemistry. 1998; 70:431-437.	
**	FULTON et al., Advanced multiplexed analysis with the FlowMetrix system. Clin Chem. 1997 Sep; 43(9):1749-56.	
**	FURKA et al., General method for rapid synthesis of multicomponent peptide mixtures. Int J Pept Protein Res. 1991 Jun;37(6):487-93.	
**	GEBEL, Molecule counting made easy. Anal Chem. 2009 Sept. 1; 7130-7131.	
**	GIAEVER et al., Micromotion of mammalian cells measured electrically. Proc Natl Acad Sci U S A. 1991 Sep 1;88(17):7896-900.	
**	GOEDERT et al., Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. Neuron. 1989 Oct;3(4):519-26.	

EXAMINER:	DATE CONSIDERED:
-----------	------------------

* EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609: Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

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7510714.1

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				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
Sheet	20	of	28				

**	GORRIS et al., Analytical chemistry on the femtoliter scale. Angew Chem Int Ed. 2010; 49:2-18.	
**	GORRIS et al., Mechanistic aspects of horseradish peroxidase elucidated through single-molecule studies. J Am Chem Soc. 2009 May 6; 131(17):6277-82.	
**	GORRIS et al., Optical-fiber bundles. FEBS J. 2007 Nov; 274(21):5462-70. Epub 2007 Oct 12.	
**	GORRIS et al., Stochastic inhibitor release and binding from single-enzyme molecules. Proc Natl Acad Sci U S A. 2007 Nov 6; 104(45):17680-5. Epub 2007 Oct 26.	
**	GUGLIELMOTTO et al., The up-regulation of BACE1 mediated by hypoxia and ischemic injury: role of oxidative stress and HIF1 α . J Neurochem. 2009;108:1045-56.	
**	HÄRMA et al., Europium nanoparticles and time-resolved fluorescence for ultrasensitive detection of prostate-specific antigen. Clin Chem. 2001 Mar; 47(3):561-8.	
**	HÄRMA et al., Miniature single-particle immunoassay for prostate-specific antigen in serum using recombinant Fab fragments. Clin Chem. 2000 Nov; 46(11):1755-61.	
**	HÄRMA et al., Zeptomole detection sensitivity of prostate-specific antigen in a rapid microtitre plate assay using time-resolved fluorescence. Luminescence. 2000 Nov-Dec;15(6):351-5.	
**	HASHIDA et al., Immune complex transfer enzyme immunoassay that is more sensitive and specific than western blotting for detection of antibody immunoglobulin G to human immunodeficiency virus type 1 in serum with recombinant pol and gag proteins as antigens. Clin Diagn Lab Immunol. 1995 Sep; 2(5):535-41.	
**	HAUGLAND, Handbook: A Guide to Fluorescent Probes and Labeling Technologies. Invitrogen, Eugene, OR. Molecular Probes, US. 2005. Pages 473-538.	
**	HE et al., Selective encapsulation of single cells and subcellular organelles into picoliter- and femtoliter-volume droplets. Anal Chem. 2005 Mar 15; 77(6):1539-44.	
**	HEALEY et al., Fiberoptic DNA sensor array capable of detecting point mutations. Anal Biochem. 1997 Sep 5; 251(2):270-9.	
**	HEALEY et al., Multianalyte biosensors on optical imaging bundles. Biosens Bioelectron. 1997; 12(6):521-9.	
**	HEALEY et al., Photodeposition of micrometer-scale polymer patterns on optical imaging fibers. Science. 1995 Aug 25; 269(5227):1078-80.	
**	HINDSON et al., High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. Anal Chem. 2011 Nov 15;83(22):8604-10. Epub 2011 Oct 28.	
**	HIRANO et al., A novel method for DNA molecular counting. Nucleic Acids Symp Ser. 2000;(44):157-8.	
**	HIRSCHFELD, Remote and in-situ analysis. Anal Chem. 1986; 324:618-624.	
**	HUNSAKER et al., Nucleic acid hybridization assays employing dA-tailed capture probes. II. Advanced multiple capture methods. Anal Biochem. 1989 Sep; 181(2):360-70.	
**	JENDROSKA et al., Ischemic stress induces deposition of amyloid β immunoreactivity in human brain. Acta Neuropathol. 1995;90:461-6.	

EXAMINER:	DATE CONSIDERED:
-----------	------------------

* EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609: Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

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7510714.1

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				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
Sheet	21	of	28				

**	JEROMIN et al., Ultrasensitive detection of neurodegenerative biomarkers in blood with the fully automated Simoa analyzer. AAIC Meeting, Copenhagen, Denmark. Abstract and Poster. 2014. 2 pages.	
**	JEROMIN et al., Ultrasensitive detection of total tau in blood in sport-related concussion with the fully automated Simoa analyzer. Traumatic Brain Injury Conference. Washington, DC. Abstract and Poster. 2014. 2 pages.	
**	JOHNSON et al., Identification of multiple analytes using an optical sensor array and pattern recognition neural networks. Analytical Chemistry. 1997; 69(22):4641-4648.	
**	JOOS, Quanterix Web Symposium: Immunoassays in Multiplex for Biomarker Discovery and Validation. Presentation. 2013 Feb 27. 43 pages.	
**	KE et al., Improving precision of proximity ligation assay by amplified single molecule detection. PLoS One. 2013 Jul 16;8(7):e69813. doi: 10.1371/journal.pone.0069813. 5 pages.	
**	KISS et al., High-throughput quantitative polymerase chain reaction in picoliter droplets. Anal Chem. 2008 Dec 1;80(23):8975-81.	
**	KREMSKY et al., Immobilization of DNA via oligonucleotides containing an aldehyde or carboxylic acid group at the 5' terminus. Nucleic Acids Res. 1987 Apr 10; 15(7):2891-909.	
**	KUANG et al., Living bacterial cell array for genotoxin monitoring. Anal Chem. 2004 May 15; 76(10):2902-9.	
**	KUANG et al., Monitoring "promiscuous" drug effects on single cells of multiple cell types. Anal Biochem. 2005 Oct 15; 345(2):320-5.	
**	KUANG et al., Simultaneously monitoring gene expression kinetics and genetic noise in single cells by optical well arrays. Anal Chem. 2004 Nov 1; 76(21):6282-6.	
**	LAFRATTA et al., Very high density sensing arrays. Chem Rev. 2008 Feb; 108(2):614-37. Epub 2008 Jan 30.	
**	LEE et al., A fiber-optic microarray biosensor using aptamers as receptors. Anal Biochem. 2000 Jun 15; 282(1):142-6.	
**	LI et al., Detection of single-molecule DNA hybridization using enzymatic amplification in an array of femtoliter-sized reaction vessels. J Am Chem Soc. 2008 Sep 24; 130(38):12622-3. Epub 2008 Sep 3.	
**	LI et al., Hypoxia increases A β generation by altering β - and γ -cleavage of APP. Neurobiol Aging. 2009;30:1091-8.	
**	LI et al., Molecule direct and quantitative counting of antibody-protein complexes in solution. Anal Chem. 2004 Aug 1; 76(15):4446-51.	
**	LU et al., Single-molecule enzymatic dynamics. Science. 1998 Dec 4; 282(5395):1877-82.	
***	LUI et al., Plasma amyloid-beta as a biomarker in Alzheimer's disease: the AIBL study of aging. J Alzheimers Dis. 2010;20(4):1233-42. doi: 10.3233/JAD-2010-090249.	

EXAMINER:	DATE CONSIDERED:
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				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
Sheet	22	of	28				

**	LUO et al., Single-molecule and ensemble fluorescence assays for a functionally important conformational change in T7 DNA polymerase. <i>Proc Natl Acad Sci U S A</i> . 2007 Jul 31; 104(31):12610-5. Epub 2007 Jul 18.	
**	MELIN et al., Microfluidic large-scale integration: the evolution of design rules for biological automation. <i>Annu Rev Biophys Biomol Struct</i> . 2007; 36:213-31.	
**	MICHAEL et al., Combined imaging and chemical sensing of fertilization-induced acid release from single sea urchin eggs. <i>Anal Biochem</i> . 1999 Sep 10; 273(2):168-78.	
**	MICHAEL et al., Randomly ordered addressable high-density optical sensor arrays. <i>Anal Chem</i> . 1998 Apr 1; 70(7):1242-8.	
**	MONDELLO et al., CSF and Plasma Amyloid- β Temporal Profiles and Relationships With Neurological Status and Mortality After Severe Traumatic Brain Injury: Results of a Pilot Study. 26th Annual Congress of the European Society of Intensive Care Medicine. Abstract. 2013. 2 pages.	
**	MONDELLO et al., CSF and Plasma Amyloid- β Temporal Profiles and Relationships with Neurological Status and Mortality after Severe Traumatic Brain Injury. <i>Scientific Reports</i> . 2014 Oct 10;4:6446. doi:10.1038/srep06446. 6 pages.	
**	MONK et al., Fabrication of gold microtubes and microwires in high aspect ratio capillary arrays. <i>J Am Chem Soc</i> . 2004 Sep 22; 126(37):11416-7.	
**	MONK et al., Optical fiber-based biosensors. <i>Anal Bioanal Chem</i> . 2004 Aug; 379(7-8):931-45. Epub 2004 Jun 23.	
**	MONK et al., Progress toward the determination of Sr^{2+} in highly basic solutions using imaging optical fiber sensor arrays. <i>J. Mater. Chem</i> . 2005; 15:4361-4366.	
**	MORRISON et al., Nanoliter high throughput quantitative PCR. <i>Nucleic Acids Res</i> . 2006;34(18):e123. Epub 2006 Sep 25.	
***	MORTBERG, Assessment of the Cerebral Ischemic/Reperfusion Injury after Cardiac Arrest. <i>Acta Universitatis Upsaliensis Uppsala</i> . Dissertation. 2010. 71 pages.	
**	MUNKHOLM et al., Polymer modification of fiber optic chemical sensors as a method of enhancing fluorescence signal for pH measurement. <i>Anal Chem</i> . 1986; 58:1427-1430.	
**	NAGAI et al., High-throughput PCR in silicon based microchamber array. <i>Biosens Bioelectron</i> . 2001 Dec; 16(9-12):1015-9.	
**	NALEFSKI et al., Single-molecule detection for femtomolar quantification of proteins in heterogeneous immunoassays. <i>Clin Chem</i> . 2006 Nov; 52(11):2172-5.	
**	NAM et al., Nanoparticle-based bio-bar codes for the ultrasensitive detection of proteins. <i>Science</i> . 2003 Sep 26;301(5641):1884-6.	
**	NESELIUS et al., Olympic boxing is associated with elevated levels of the neuronal protein tau in plasma. <i>Brain Inj</i> . 2013;27(4):425-33. doi: 10.3109/02699052.2012.750752. Epub 2013 Mar 8.	

EXAMINER:	DATE CONSIDERED:
-----------	------------------

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Sheet	23	of	28				

**	NIEMEYER et al., Combination of DNA-directed immobilization and immuno-PCR: very sensitive antigen detection by means of self-assembled DNA-protein conjugates. Nucleic Acids Res. 2003 Aug 15; 31(16):e90.	
**	OKRONGLY, Single Molecule Enzyme Detection and Application to Immunoassay: Implications for Personalized Medicine. Abstract and Presentation. ISE International Conference. 2010 May 4. 24 pages.	
**	PANOVA et al., In situ fluorescence imaging of localized corrosion with a pH-sensitive imaging fiber. Anal Chem. 1997 Apr 15; 69(8):1635-41.	
**	PANTANO et al., Analytical applications of optical imaging fibers. Anal Chem. 1995 Aug 1; 67(15):481A-487A.	
**	PANTANO et al., Ordered nanowell arrays. Chemistry of Materials. 1996;8: 2832-2835.	
**	PANTANO et al., Toward a near-field optical array. Rev. Sci. Instrum. 1997; 68(3) 1357-1359	
**	PETERSON et al., Fiber optic pH probe for physiological use. Anal Chem. 1980 May; 52(6):864-9.	
**	PRABHAKAR et al., Simultaneous quantification of proinflammatory cytokines in human plasma using the LabMAP assay. J Immunol Methods. 2002 Feb 1;260(1-2):207-18.	
**	QIU et al., Fluorescence single-molecule counting assays for high-sensitivity detection of cytokines and chemokines. Clin Chem. 2007 Nov; 53(11):2010-2.	
**	RANDALL et al., Plasma Tau Levels in Concussed Hockey Players. Neuroscience Conference, San Diego, CA. Abstract and Poster. 2013. 2 pages	
**	RANDALL et al., Serum Tau is predictive of 6-month cognitive outcome following cardiac arrest. 2011 Neuroscience Conference. Washington, DC. Abstract and Poster. 2011. 2 pages.	
**	RANDALL et al., Tau proteins in serum predict neurological outcome after hypoxic brain injury from cardiac arrest: Results of a pilot study. Resuscitation. 2013 Mar;84(3):351-6. doi: 10.1016/j.resuscitation.2012.07.027. Epub 2012 Aug 9. Epub 2012 Aug 9. 6 pages.	
**	RANDLE et al., Integrating molecular detection and response to create self-signalling antibodies. Biochem Biophys Res Commun. 2004 Nov 12; 324(2):504-10.	
**	RISSIN et al., Attomolar detection of proteins in serum using single molecule enzyme-linked immunosorbent assays. Quanterix Corporation. Oak Ridge Conference, San Jose, CA. Poster. 2010. 1 page.	
**	RISSIN et al., Digital concentration readout of single enzyme molecules using femtoliter arrays and Poisson statistics. Nano Lett. 2006 Mar; 6(3):520-3.	
**	RISSIN et al., Digital readout of target binding with attomole detection limits via enzyme amplification in femtoliter arrays. J Am Chem Soc. 2006 May 17; 128(19):6286-7.	
**	RISSIN et al., Distinct and long-lived activity states of single enzyme molecules. J Am Chem Soc. 2008 Apr 16; 130(15):5349-53. Epub 2008 Mar 5.	
**	RISSIN et al., Duplexed sandwich immunoassays on a fiber-optic microarray. Anal Chim Acta. 2006 Mar 30; 564(1):34-9. Epub 2005 Nov 11.	

EXAMINER:	DATE CONSIDERED:
-----------	------------------

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Sheet	24	of	28				

**	RISSIN et al., Immunoassays with broad dynamic ranges based on combining digital and digitally-enhanced analog detection of enzyme labels. Oak Ridge Conference. Poster 7 and Abstract. 2011 Apr 14-15. 2 pages.	
**	RISSIN et al., Multiplexed single molecule immunoassays. Lab Chip. 2013 Aug 7;13(15):2902-11. doi: 10.1039/c3lc50416f.	
**	RISSIN et al., Simultaneous detection of single molecules and singulated ensembles of molecules enables immunoassays with broad dynamic range. Anal Chem. 2011 Mar 15;83(6):2279-85. Epub 2011 Feb 23.	
**	RISSIN et al., Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotechnol. 2010 Jun; 28(6):595-9 and supplemental pages. Epub 2010 May 23.	
**	ROEFFAERS et al., Single-molecule fluorescence spectroscopy in (bio)catalysis. Proc Natl Acad Sci U S A. 2007 Jul 31; 104(31):12603-9. Epub 2007 Jul 30.	
**	RONDELEZ et al., Highly coupled ATP synthesis by F1-ATPase single molecules. Nature. 2005 Feb 17; 433(7027):773-7.	
**	RONDELEZ et al., Microfabricated arrays of femtoliter chambers allow single molecule enzymology. Nat Biotechnol. 2005 Mar; 23(3):361-5. Epub 2005 Feb 20.	
**	ROTMAN, Measurement of activity of single molecules of beta-D-galactosidase. Proc Natl Acad Sci U S A. 1961 Dec 15; 47:1981-91.	
**	SCHAUER et al., A cross-reactive, class-selective enzymatic array assay. J Am Chem Soc. 2001 Sep 26; 123(38):9443-4.	
**	SCHMIDINGER, et al., Inhibitor and protein microarrays for activity-based recognition of lipolytic enzymes. Chembiochem. 2006 Mar; 7(3):527-34.	
**	SCHWEITZER et al., Inaugural article: immunoassays with rolling circle DNA amplification: a versatile platform for ultrasensitive antigen detection. Proc Natl Acad Sci U S A. 2000 Aug 29; 97(18):10113-9.	
**	SEYDACK, Nanoparticle labels in immunosensing using optical detection methods. Biosens Bioelectron. 2005 Jun 15; 20(12):2454-69. Epub 2004 Dec 16.	
**	SHAHIM et al., Blood biomarkers for brain injury in concussed professional ice hockey players. JAMA Neurol. 2014 Jun;71(6):684-92. doi:10.1001/jamaneuro.2014.367. Epub 2014 Mar 13. 9 pages.	
**	SHEN et al. High-throughput SNP genotyping on universal bead arrays. Mutat Res. 2005 Jun 3;573(1-2):70-82.	
**	SHEPHARD et al., Array-based binary analysis for bacterial typing. Anal Chem. 2005 Jan 1; 77(1):319-26.	
**	SHIYA et al., Tau protein in the cerebrospinal fluid is a marker of brain injury after aortic surgery. Ann Thorac Surg. 2004 Jun;77(6):2034-8.	
**	SONG et al., Detecting biological warfare agents. Emerg Infect Dis. 2005 Oct; 11(10):1629-32.	

EXAMINER:	DATE CONSIDERED:
-----------	------------------

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Sheet	25	of	28				

**	SONG et al., Direct Detection of Bacterial DNA and viral RNA at Subfemtomolar Concentrations Using Single Molecule Arrays (Simoa). 2013 Oakridge Conference. Baltimore, MD. Abstract and Poster. 2013. 2 pages.	
**	SONG et al., Direct detection of bacterial genomic DNA at sub-femtomolar concentrations using single molecule arrays. Anal Chem. 2013 Feb 5;85(3):1932-9. doi: 10.1021/ac303426b. Epub 2013 Jan 18. Supporting information included.	
**	SONG et al., Fiber-optic microsphere-based arrays for multiplexed biological warfare agent detection. Anal Chem. 2006 Feb 15; 78(4):1023-33.	
**	SONG et al., Single molecule measurements of tumor necrosis factor α and interleukin-6 in the plasma of patients with Crohn's disease. J Immunol Methods 2011 Sep 30;372(1-2):177-86. Epub 2011 Jul 27.	
**	SOUKKA et al., Supersensitive time-resolved immunofluorometric assay of free prostate-specific antigen with nanoparticle label technology. Clin Chem. 2001; 47(7):1269-78.	
**	STAMOU et al., Self-assembled microarrays of attoliter molecular vessels. Angew Chem Int Ed Engl. 2003 Nov 24; 42(45):5580-3.	
**	STEEMERS et al., Multi- analyte sensing: from site-selective deposition to randomly ordered addressable optical sensors. Microchimica Acta. 1999; 131:99-105.	
**	STEEMERS et al., Screening unlabeled DNA targets with randomly ordered fiber-optic gene arrays. Nat Biotechnol. 2000 Jan; 18(1):91-4.	
**	STITZEL et al., Array-to-array transfer of an artificial nose classifier. Anal Chem. 2001 Nov 1; 73(21):5266-71.	
**	SUBBARAMAN, Detecting single cancer molecules. Technol Rev. 2010 Jun 3. Last accessed at http://www.technologyreview.com/biomedicine/25462/ on 1/31/2012. 2 pages.	
**	SUH et al., Hypoxic ischemia and proteasome dysfunction alter tau isoform ratio by inhibiting exon 10 splicing. J Neurochem. 2010 Jul;114(1):160-70. doi: 10.1111/j.1471-4159.2010.06732.x. Epub 2010 Apr 3.	
**	SUN et al., Hypoxia facilitates Alzheimer's disease pathogenesis by up-regulating BACE1 gene expression. PNAS. 2006 Dec 5;103(49):18727-32.	
**	SYKES et al., Quantitation of targets for PCR by use of limiting dilution. Biotechniques. 1992;13(3):444-9.	
**	SZUNERITS et al., "Aluminum Surface Corrosion and the Mechanism of Inhibitors Using pH and Metal Ion Selective Imaging Fiber Bundles," Analytical Chemistry, 2002, 74(4), 886-894.	
**	SZUNERITS et al., "Fabrication of an Optoelectrochemical Microring Array," Analytical Chemistry, 2002, 74(7), 1718-1723.	
**	SZUNERITS et al., Spatially resolved electrochemiluminescence on an array of electrode tips. Anal Chem. 2003 Sep 1; 75(17):4382-8.	
**	SZUNERITS et al., The use of optical fiber bundles combined with electrochemistry for chemical imaging. Chemphyschem. 2003 Feb 17; 4(2):186-92.	

EXAMINER:	DATE CONSIDERED:
-----------	------------------

* EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609: Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

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				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
Sheet	26	of	28				

**	SZURDOKI et al., A duplexed microsphere-based fluorescent immunoassay. Anal Biochem. 2001 Apr 15; 291(2):219-28.	
**	TAM et al., An imaging fiber-based optical tweezer array for microparticle array assembly. Applied Physics Letters. 2004; 84(21):4289-4291.	
**	TAM et al., Fabrication and optical characterization of imaging fiber-based nanoarrays. Talanta. 2005 Sep 15; 67(3):498-502. Epub 2005 Jul 27.	
**	TAM et al., Parallel microparticle manipulation using an imaging fiber bundle-based optical tweezer array and a digital micromirror device. Applied Physics Letters. 2006; 89:194101/1-194101/3.	
**	TAN et al., Monitoring the reactions of single enzyme molecules and single metal ions. Anal. Chem. 1997; 69:4242-4248.	
**	TANEN et al., Development of an Ultrasensitive Digital Immunoassay on the Single Molecule Array (Simoa™) Platform. 2014 AAPS Annual Meeting, San Diego, CA. Abstract and Poster. 2014 Nov 2-6, 2 pages.	
**	TAYLOR et al., Application of high-density optical microwell arrays in a live-cell biosensing system. Anal Biochem. 2000 Feb 15; 278(2):132-42.	
**	TAYLOR et al., Hypoxic Enhancement of Quantal Catecholamine Secretion: Evidence for the Involvement of Amyloid β -Peptides. J Biol Chem. 1999 Oct 29;274(44):31217-22.	
**	TESSLER et al., Protein quantification in complex mixtures by solid phase single-molecule counting. Anal Chem. 2009 Sep 1; 81(17):7141-8.	
**	TETTENBORN et al., Prophylaxis and acute therapy of arterial embolism with special reference to cerebral embolism. Herz. 1991 Dec;16(6):444-55. Review. Abstract only. 1 page.	
**	TIMMERMAN, Quanterix CEO sets sight on early detection of cancer, neurological diseases in the blood. Xconomy. 2010 Jan 19. Last accessed at http://www.xconomy.com/boston/2010/01/19/quanterix-ceo-sets-sight-on-early-detection-of-cancer-neurological-diseases-in-the-blood/ on 1/31/2012. 4 pages.	
**	TODD et al., Ultrasensitive flow-based immunoassays using single-molecule counting. Clin Chem. 2007 Nov; 53(11):1990-5. Epub 2007 Sep 21.	
**	TROMBERG et al., Development of antibody-based fiber-optic sensors for detection of a benzo[a]pyrene metabolite. Anal Chem. 1988 Sep 15; 60(18):1901-8.	
**	UEBERFELD et al., Reversible ratiometric probe for quantitative DNA measurements. Anal Chem. 2004 Feb 15; 76(4):947-52.	
**	VO-DINH et al., Phase-resolved fiber-optics fluorosensor. Applied Spectroscopy. 1990; 44(1):128-132.	
**	VOGELSTEIN et al., Digital PCR. Proc Natl Acad Sci U S A. 1999 Aug 3;96(16):9236-41.	
**	WALT et al., Biosensing with live cells using a high-density optical fiber array. Radiation Research. 2001; 156(4):442.	

EXAMINER:	DATE CONSIDERED:
-----------	------------------

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Sheet	27	of	28				

**	WALT et al., Microsensor arrays for saliva diagnostics. Ann N Y Acad Sci. 2007 Mar; 1098:389-400.	
**	WALT et al., Optical sensor arrays for odor recognition. Biosens Bioelectron. 1998 Sep 15; 13(6):697-9.	
**	WALT et al., Ultrasensitive detection of proteins using single molecule arrays (SiMoA). Presented March 1, 2010. Pittcon. Abstract and PowerPoint presentation. 33 pages.	
**	WALT, An array of solutions, fiber arrays contribute to studies of individual cellular behavior and response. SPIE'S oemagazine. 2005; 19-21.	
**	WALT, Fiber optic array biosensors. Biotechniques. 2006 Nov; 41(5):529, 531, 533 passim.	
**	WALT, Fiber optic imaging sensors. Accounts of Chemical Research. 1998; 31:267-278.	
**	WALT, Imaging optical sensor arrays. Curr Opin Chem Biol. 2002 Oct; 6(5):689-95.	
**	WALT, Optical methods for single molecule detection and analysis. Anal Chem. 2013 Feb 5;85(3):1258-63. doi: 10.1021/ac3027178. Epub 2012 Dec 19.	
**	WALT, Techview: molecular biology. Bead-based fiber-optic arrays. Science. 2000 Jan 21; 287(5452):451-2.	
**	WANG et al., Quantification of protein based on single-molecule counting by total internal reflection fluorescence microscopy with adsorption equilibrium. Anal Chim Acta. 2007 May 2; 590(1):104-9. Epub 2007 Mar 15.	
**	WARREN et al., Transcription factor profiling in individual hematopoietic progenitors by digital RT-PCR. Proc Natl Acad Sci U S A. 2006 Nov 21;103(47):17807-12. Epub 2006 Nov 10.	
**	WHITAKER et al., Fiber-based single cell analysis of reporter gene expression in yeast two-hybrid systems. Anal Biochem. 2007 Jan 1; 360(1):63-74. Epub 2006 Oct 30.	
**	WHITAKER et al., Multianalyte single-cell analysis with multiple cell lines using a fiber-optic array. Anal Chem. 2007 Dec 1; 79(23):9045-53. Epub 2007 Nov 1.	
**	WHITE et al., An olfactory neuronal network for vapor recognition in an artificial nose. Biol Cybern. 1998 Apr; 78(4):245-51.	
**	WHITE et al., Rapid analyte recognition in a device based on optical sensors and the olfactory system. Analytical Chemistry. 1996; 68(13):2191-2202.	
**	WILSON et al., Development of AccuPSA™, a novel digital immunoassay for sub-femtomolar measurement of PSA in post radical prostatectomy patients. AACR Molecular diagnostics in Cancer Therapeutic Development Poster. 2011. 1 page.	
**	WILSON et al., Fifth-generation digital immunoassay for prostate-specific antigen by single molecule array technology. Clin Chem. 2011 Dec;57(12):1712-21. Epub 2011 Oct 13.	
**	WILSON et al., Simoa™ HD-1: a fully automated digital immunoassay analyzer capable of single molecule counting, sub-femtomolar sensitivity, and multiplexing. 2014 AACC Meeting. Chicago, IL. Abstract and Poster. 2014. 2 pages.	

EXAMINER:	DATE CONSIDERED:
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				GROUP ART UNIT: 1649		EXAMINER: Not Yet Assigned	
Sheet	28	of	28				

**	WILSON, Serum Measurement of Hypoxia-Induced Amyloid Beta 1-42 Following Resuscitation from Cardiac Arrest. Abstract and Poster. American Academy of Neurology Annual Meeting. 2011 Apr 9. 2 pages.	
**	WU et al., Development and preliminary clinical validation of a high sensitivity assay for cardiac troponin using a capillary flow (single molecule) fluorescence detector. Clin Chem. 2006 Nov; 52(11):2157-9.	
***	WUNDERLICH et al., Neuron-specific enolase and tau protein as neurobiochemical markers of neuronal damage are related to early clinical course and long-term outcome in acute ischemic stroke. Clinical Neurology and Neurosurgery. 2006 Sep;108:558-63.	
**	XIE et al., Optical studies of single molecules at room temperature. Annu Rev Phys Chem. 1998; 49:441-80.	
**	XIE et al., Single gold nanoparticles counter: an ultrasensitive detection platform for one-step homogeneous immunoassays and DNA hybridization assays. J Am Chem Soc. 2009 Sep 9;131(35):12763-70.	
**	XUE et al., Differences in the chemical reactivity of individual molecules of an enzyme. Nature. 1995 Feb 23; 373(6516):681-3.	
**	YAN et al., Analyzing polyubiquitin chains upon ubiquitin activating enzyme inhibition from cell culture & tumor lysates using the Quanterix's single molecule array (Simoa) technology. 2013 Society for the Laboratory Automation & Screening Annual Meeting, Orlando, FL. Abstract and Poster. 2013. 2 pages.	
**	YOUNG et al., Integrating high-content screening and ligand-target prediction to identify mechanism of action. Nat Chem Biol. 2008 Jan; 4(1):59-68. Epub 2007 Dec 9.	
**	ZETTERBERG et al., Hypoxia due to cardiac arrest induces a time-dependent increase in serum amyloid β levels in humans. PLoS One. 2011;6(12):e28263. doi: 10.1371/journal.pone.0028263. Epub 2011 Dec 14.	
**	ZETTERBERG et al., Hypoxia due to cardiac arrest induces a time-dependent increase in serum amyloid β levels in humans. PLoS One. 2011;6(12):e28263. Epub 2011 Dec 14.	
**	ZETTERBERG et al., Plasma tau levels in Alzheimer's disease. Alzheimers Res Ther. 2013 Mar 28;5(2):9. doi: 10.1186/alzrt163. eCollection 2013.	
**	ZETTERBERG, Quanterix Web Symposium: Peripheral Blood Biomarkers for Brain Damage – Challenging but Feasible. Presentation. 2012 Oct 16. 29 pages.	
**	ZHANG et al., Hypoxia-inducible Factor 1 α (HIF-1 α)-mediated Hypoxia Increases BACE1 Expression and β -Amyloid Generation. J Biol Chem. 2007 Apr 13;282(15):10873-80.	

[NOTE – No copies of U.S. patents, published U.S. patent applications, or pending, unpublished patent applications stored in the USPTO's Image File Wrapper (IFW) system, are included. See 37 CFR § 1.98 and 1287OG163. Copies of all other patent(s), publication(s), unpublished, pending U.S. patent applications, or other information listed are provided as required by 37 CFR § 1.98 unless 1) such copies were provided in an IDS in an earlier application that complies with 37 CFR § 1.98, and 2) the earlier application is relied upon for an earlier filing date under 35 U.S.C. § 120.]

EXAMINER:	DATE CONSIDERED:
/Adam Weidner/	03/11/2021

* EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609: Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

**a copy of this reference is not provided as it was previously cited by or submitted to the office in a prior application, Serial No. 14/111,326, filed June 24, 2014, and relied upon for an earlier filing date under 35 U.S.C. 120 (continuation, continuation-in-part, and divisional applications).

***a copy of this reference is not provided as it was previously cited by or submitted to the office in a prior application, Serial No. 15/269,142, filed September 19, 2016, and relied upon for an earlier filing date under 35 U.S.C. 120 (continuation, continuation-in-part, and divisional applications).

7510714.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /AMW/

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L4	12	L3 and tau.clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2021/03/11 15:14
L3	8,321	L1 or L2	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2021/03/11 15:14
L2	8,283	((wilson near2 david) or (john near2 zetterber) or (kaj near2 blennow) or (jeffrey near2 randall)).in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2021/03/11 15:14
L1	51	quanterix.as.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2021/03/11 15:14

EAST Search History (Interference)

<This search history is empty>

3/11/2021 3:17:35 PM

C:\Users\aweidner\Documents\EAST\Workspaces\16522237.wsp



UNITED STATES PATENT AND TRADEMARK OFFICE

USPTO Automated Interview Request (AIR)

Jun 22 2021

This paper requesting to schedule and/or conduct an interview is appropriate because:

This submission is requested to be accepted as an authorization for this interview to communicate via the internet. Recognizing that Internet communications are not secure, I hereby authorize the USPTO to communicate with the undersigned concerning scheduling of the interview via video conference, instant messaging, or electronic mail, and to conduct the interview in accordance with office practice including video conferencing.

Name(s) :

Michael J. Pomianek, Andrew G. Maher, David C. Duffy

S-signature:

/Michael J Pomianek/

Registration Number:

46190

U.S. Application Number:

16522237

Confirmation Number:

4736

E-mail Address:

michael.pomianek@wolfgreenfield.com

Phone Number:

+1 6176468288

Proposed Time of Interview:

6-30-2021 2:30 PM ET

Alternative Proposed Time(s) of Interview:

6-29-2021 3:00 PM ET

Alternative Proposed Time(s) of Interview:

6-29-2021 4:00 PM ET

Preferred Interview Type:

Telephonic

I am the applicant or applicant's representative for this application.

Topic for Discussion:

We propose discussing the rejection of claim 92 over US20100075407 in view of Quanterix 2010 IQT Technology Focus Day (Quanterix 2010a). I will be joined by Andrew G. Maher, Reg. No. 79399 and Dr. David C. Duffy, the Chief Technology Officer of Quanterix Corporation. Dr. Duffy is a co-inventor of US20100075407 and presented the Quanterix ing the commercial/academic success the Patent Offices interpretation



UNITED STATES
PATENT AND TRADEMARK OFFICE

Docket No.: Q0052.70018US03
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: David Wilson
Application No.: 16/522,237
Confirmation No.: 4736
Filed: July 25, 2019
For: METHODS OF DETERMINING A TREATMENT PROTOCOL FOR
AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A
BRAIN INJURY
Examiner: A. M. Weidner
Art Unit: 1649

Certificate of Electronic Filing under 37 C.F.R. § 1.8	
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.8(a)(4).	
Dated: July 16, 2021	Electronic Signature for: /Eileen M. MacKenzie/

RESPONSE TO NON-FINAL OFFICE ACTION UNDER 37 C.F.R. § 1.111

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

INTRODUCTORY COMMENTS

In response to the Office Action dated March 16, 2021, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

AMENDMENTS TO THE CLAIMS

Please replace all prior versions, and listings, of claims in the application with the following list of claims:

1. - 10. (Canceled)

11. (Currently Amended) The method of claim 104 ~~[[92]]~~, wherein the brain injury results from a hypoxic event.

12. - 19. (Canceled)

20. (Currently Amended) The method claim 92, wherein the limit of detection for the analytical protein concentration measurement assay is less than about 0.02 pg/mL.

21. (Currently Amended) The method of claim 104 ~~[[92]]~~, wherein the brain injury comprises a hypoxic event caused by cardiac arrest.

22. (Currently Amended) The method of claim 104 ~~[[92]]~~, wherein the brain injury comprises a hypoxic event caused by stroke.

23. (Currently Amended) The method of claim 104 ~~[[92]]~~, wherein the brain injury comprises a hypoxic event caused by an ischemic event.

24. (Currently Amended) The method of claim 104 ~~[[92]]~~, wherein the brain injury comprises a hypoxic event caused by a thrombosis.

25. - 91. (Cancelled)

92. (Currently Amended) A method of producing a bodily body fluid sample of a patient ~~recovering from a brain injury~~ suspected of having a neurological condition containing an analytically quantified amount of endogenous tau protein, said method comprising:

(A) obtaining a volume of bodily body fluid comprising blood, or a blood component selected from plasma and serum,

(B) diluting the volume of bodily body fluid, and

(C) quantifying through the use of an analytical protein concentration measurement assay a concentration of tau protein in the volume of bodily body fluid to produce the bodily body fluid sample containing the analytically quantified amount of endogenous tau protein;

wherein the analytically quantified concentration of endogenous tau protein contained in the bodily body fluid sample is less than about 5 pg/ml, and a limit of quantification of the analytical protein concentration measurement assay used in step (C) for quantifying the concentration of tau protein is less than about 0.2 pg/mL.

93. (Currently Amended) The method of claim 92, wherein the analytical protein concentration measurement assay used in step (C) comprises:

i. exposing a plurality of capture objects that each include a binding surface having affinity for tau protein molecules to the volume of bodily body fluid;

ii. immobilizing at least a portion of the tau protein molecules with respect to the plurality of capture objects such that at least some of the capture objects associate with at least one tau protein molecule and a statistically significant fraction of the capture objects do not associate with any tau protein molecules;

iii. determining a proportion of the plurality of capture objects that associated with a tau protein molecule in step ii; and

iv. determining a measure of tau protein molecule concentration in the volume of bodily body fluid based at least in part on the proportion determined in step iii.

94. (Previously Presented) The method of claim 93, wherein the plurality of capture objects comprises a plurality of beads.

95. (Currently Amended) The method of claim 92, wherein the analytical protein concentration measurement assay used in step (C) comprises spatially segregating ~~segregated~~ tau protein molecules into a plurality of locations, wherein a statistically significant fraction of the locations contain a single tau protein molecule and a statistically significant fraction of the locations do not contain any tau protein molecules

96. (Previously Presented) The method of claim 95, wherein the plurality of locations comprises a plurality of reaction vessels.

97. (Previously Presented) The method of claim 92, wherein each of the tau protein molecules quantified in step (C) is selected from the group consisting of tau 23 (352, 0N3R), tau 24 (383, 0N4R), tau 37 (381, 1N3R), tau 34 (412, 1N4R), tau 39 (410, 2N3R), and tau 40 (441, 2N4R).

98. (Previously Presented) The method of claim 97, wherein at least a portion of the tau protein molecules are phosphorylated.

99. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid comprises blood.

100. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid comprises plasma.

101. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid comprises serum.

102. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid does not comprise cerebrospinal fluid (CSF).

103. (Currently Amended) The method of claim 104 [[92]], wherein the volume of bodily fluid has been collected from the patient within 12 hours of the brain injury.

104. (New) The method of claim 92, wherein the patient suspected of having a neurological condition is recovering from a brain injury.

105. (New) The method of claim 92, wherein the analytical protein concentration measurement assay used in step (C) comprises:

- i. suspending a plurality of capture objects that each include a binding surface having affinity for tau protein molecules in the diluted volume of bodily fluid,
- ii. immobilizing at least a portion of the tau protein molecules with respect to at least some of the plurality of capture objects suspended in the diluted volume of bodily fluid such that at least some of the capture objects associate with at least one tau protein molecule from the volume of bodily fluid,
- iii. interrogating at least some of the capture objects associated with at least one tau molecule from the volume of bodily of fluid; and
- iv. determining a measure of tau protein molecule concentration in the volume of bodily fluid based at least in part on the interrogating step performed in step iii.

106. (New) The method of claim 105, wherein the plurality of capture objects comprises a plurality of beads.

Electronic Patent Application Fee Transmittal				
Application Number:		16522237		
Filing Date:		25-Jul-2019		
Title of Invention:		METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY		
First Named Inventor/Applicant Name:		David Wilson		
Filer:		Michael Pomianek/Eileen MacKenzie		
Attorney Docket Number:		Q0052.70018US03		
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension - 1 month with \$0 paid	1251	1	220	220
Miscellaneous:				
STATUTORY OR TERMINAL DISCLAIMER	1814	2	170	340
Total in USD (\$)				560

Electronic Acknowledgement Receipt	
EFS ID:	43276981
Application Number:	16522237
International Application Number:	
Confirmation Number:	4736
Title of Invention:	METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY
First Named Inventor/Applicant Name:	David Wilson
Customer Number:	23628
Filer:	Michael Pomianek/Eileen MacKenzie
Filer Authorized By:	Michael Pomianek
Attorney Docket Number:	Q0052.70018U503
Receipt Date:	16-JUL-2021
Filing Date:	25-JUL-2019
Time Stamp:	20:07:04
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	\$ 560
RAM confirmation Number	E20217FK07241028
Deposit Account	232825
Authorized User	Eileen MacKenzie
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.17 (Patent application and reexamination processing fees)	

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	Q005270018US03-TRN-MJP.pdf	26362 0105d3b0f13204099067000ee1412d04009b08ca0	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	Q005270018US03-FEE-MJP.pdf	37729 04792da09235533d233960e06db0ee13ee0e028e0	no	1
Warnings:					
Information:					
3	Extension of Time	Q005270018US03-EXT-MJP.pdf	25375 7bfb93867753d3a059241612208232e93d009e7	no	1
Warnings:					
Information:					
4	Terminal Disclaimer Filed	Q005270018US03-TD1-AGM.pdf	71444 0195b55c10f3023d7ed386500e0600a0a0a0018	no	1
Warnings:					
Information:					
5	Terminal Disclaimer Filed	Q005270018US03-TD2-AGM.pdf	79201 05c7780051e029169940593ae97254fa5130000	no	1
Warnings:					
Information:					
6		Q005270018US03-AMN-MJP.pdf	5404366 f00ae021067a58cb9f05329b40900e96b4e56673	yes	62
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	

	Amendment/Req. Reconsideration-After Non-Final Reject	1	1				
	Claims	2	5				
	Applicant Arguments/Remarks Made in an Amendment	6	62				
Warnings:							
Information:							
7	Fee Worksheet (SB06)	fee-info.pdf	<table border="1"> <tr> <td>33196</td><td rowspan="2">no</td><td rowspan="2">2</td></tr> <tr> <td>c0232a067082e7a0864e412d602ff1d15e00e92</td></tr> </table>	33196	no	2	c0232a067082e7a0864e412d602ff1d15e00e92
33196	no	2					
c0232a067082e7a0864e412d602ff1d15e00e92							
Warnings:							
Information:							
Total Files Size (in bytes):			5677673				
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.							

TRANSMITTAL FORM <i>(to be used for all correspondence after initial filing)</i>	Application Number	16/522,237-Conf. #4736
	Filing Date	July 25, 2019
	First Named Inventor	David Wilson
	Art Unit	1649
	Examiner Name	A. M. Weidner
Total Number of Pages in This Submission	Attorney Docket Number	Q0052.70018US03

ENCLOSURES (Check all that apply)		
☒ Fee Transmittal Form ☐ Fee Attached ☒ Amendment/Reply ☐ After Final ☐ Affidavits/declaration(s) ☒ Extension of Time Request ☐ Express Abandonment Request ☐ Information Disclosure Statement ☐ Certified Copy of Priority Document(s) ☐ Reply to Missing Parts/ Incomplete Application ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53	☐ Drawing(s) ☐ Licensing-related Papers ☐ Petition ☐ Petition to Convert to a Provisional Application ☐ Power of Attorney, Revocation Change of Correspondence Address ☒ Terminal Disclaimers ☐ Request for Refund ☐ CD, Number of CD(s) _____ ☐ Landscape Table on CD	☐ After Allowance Communication to TC ☐ Appeal Communication to Board of Appeals and Interferences ☐ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) ☐ Proprietary Information ☐ Status Letter ☒ Other Enclosure(s) (please Identify below): Appendices 1-4
Remarks		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT			
Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Michael J. Pomianek/		
Printed name	Michael J. Pomianek		
Date	July 16, 2021	Reg. No.	46,190

Certificate of Electronic Filing under 37 C.F.R. § 1.8	
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).	
Dated: July 16, 2021	Electronic Signature for: /Eileen M. MacKenzie/

FEE TRANSMITTAL		Complete if known	
☐ Applicant asserts small entity status. See 37 CFR 1.27.		Application Number	16/522,237-Conf. #4736
☐ Applicant certifies micro entity status. See 37 CFR 1.29. Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.		Filing Date	July 25, 2019
		First Named Inventor	David Wilson
		Examiner Name	A. M. Weidner
		Art Unit	1649
TOTAL AMOUNT OF PAYMENT	(\$560.00)	Practitioner Docket No.	Q0052.70018US03

METHOD OF PAYMENT (check all that apply)

☐ Check
 ☒ Credit Card
 ☐ Money Order
 ☐ None
 ☐ Other (please identify): _____

☒ Deposit Account
 Deposit Account Number: 23/2825
 Deposit Account Name: Wolf, Greenfield & Sacks, P.C.

For the above-identified deposit account, the Director is hereby authorized to (check all that apply):

☐ Charge fee(s) indicated below
 ☐ Charge fee(s) indicated below, **except for the filing fee**

☒ Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17, **except for any excess claims fees or multiple dependent claim fee**
 ☐ Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17

☒ Credit any overpayment of fee(s)

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES (U = undiscounted fee; S = small entity fee; M = micro entity fee)

Application Type	FILING FEES			SEARCH FEES			EXAMINATION FEES			Fees Paid (\$)
	U (\$)	S (\$)	M (\$)	U (\$)	S (\$)	M (\$)	U (\$)	S (\$)	M (\$)	
Utility	320	160*	80	700	350	175	800	400	200	
Design	220	110	55	160	80	40	640	320	160	
Plant	220	110	55	440	220	110	660	330	165	
Reissue	320	160	80	700	350	175	2,320	1,160	580	
Provisional	300	150	75	0	0	0	0	0	0	

* The \$160 small entity status filing fee for a utility application is further reduced to \$80 for a small entity status applicant who files the application via EFS-Web.

2. EXCESS CLAIM FEES

Fee Description	Undiscounted Fee (\$)	Small Entity Fee (\$)	Micro Entity Fee (\$)
Each claim over 20 (including Reissues)	100	50	25
Each independent claim over 3 (including Reissues)	480	240	120
Multiple dependent claims	860	430	215

Total Claims **Extra Claims** **Fee (\$)** **Fee Paid (\$)** **Multiple Dependent Claims**
 _____ - 20 or HP = _____ x _____ = _____ **Fee (\$)** **Fee Paid (\$)**
 HP = highest number of total claims paid for, if greater than 20.

Indep. Claims **Extra Claims** **Fee (\$)** **Fee Paid (\$)**
 _____ - 3 or HP = _____ x _____ = _____
 HP = highest number of independent claims paid for, if greater than 3.

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is \$420 (\$210 for small entity) (\$105 for micro entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee (\$)	Fee Paid (\$)
_____	_____	_____	_____	_____

_____ - 100 = _____ / 50 = _____ (round up to a whole number) x _____ = _____

4. OTHER FEE(S)

	Fees Paid (\$)
Non-English specification, \$130 fee (no small or micro entity discount)	
Non-electronic filing fee under 37 CFR 1.16(i) for a utility application, \$400 fee (\$200 small or micro entity)	
Other (e.g., late filing surcharge): 1251 Extension for response within first month	220.00
1814 Statutory disclaimer, including terminal ...	340.00

SUBMITTED BY

Signature	/Michael J. Pomianek/	Registration No. (Attorney/Agent)	46,190	Telephone	617.646.8000
Name (Print/Type)	Michael J. Pomianek	Date	July 16, 2021		

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: July 16, 2021 Electronic Signature for: /Eileen M. MacKenzie/

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)		Docket Number (Optional) Q0052.70018US03	
Application Number 16/522,237-Conf. #4736		Filed July 25, 2019	
For METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY			
Art Unit 1649		Examiner A. M. Weidner	
This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application. The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):			
	<u>Fee</u>	<u>Small Entity Fee</u>	<u>Micro Entity Fee</u>
☒ One month (37 CFR 1.17(a)(1))	\$220	\$110	\$55
☐ Two months (37 CFR 1.17(a)(2))	\$640	\$320	\$160
☐ Three months (37 CFR 1.17(a)(3))	\$1,480	\$740	\$370
☐ Four months (37 CFR 1.17(a)(4))	\$2,320	\$1,160	\$580
☐ Five months (37 CFR 1.17(a)(5))	\$3,160	\$1,580	\$790
☐ Applicant asserts small entity status. See 37 CFR 1.27. ☐ Applicant certifies micro entity status. See 37 CFR 1.29. Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously. ☐ A check in the amount of the fee is enclosed. ☒ Payment by credit card. Form PTO-2038 is attached. ☐ The Director has already been authorized to charge fees in this application to a Deposit Account. ☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number <u>23/2825</u> . ☒ Payment made via EFS-Web.			
WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.			
I am the			
☐ applicant.			
☒ attorney or agent of record. Registration number <u>46,190</u>			
☐ attorney or agent acting under 37 CFR 1.34. Registration number _____			
_____ /Michael J. Pomianek/ Signature		_____ July 16, 2021 Date	
_____ Michael J. Pomianek Typed or printed name		_____ 617.646.8000 Telephone Number	
NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.			

☐ * Total of 1 forms are submitted.

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: July 16, 2021

Electronic Signature for: /Eileen M. MacKenzie/

REMARKS

Applicant respectfully requests reconsideration. Claims 11, 20-24 and 92-103 were previously pending in this application. Claims 11, 20-24, 92-93, 95, and 103 have been amended. New claims 104-106 have been added and are supported by the specification at, for example, p. 26 line 31 to p. 27 line 17, p. 30 line 6 to p. 31 line 4, and p. 47 line 32 to p. 48 line 25. No new matter has been added.

Claims 11, 20-24 and 92-106 are now pending in this application with claim 92 being the sole independent claim.

Interview Summary

The Applicant's Representatives, Michael J. Pomianek, Ph.D., Reg. No.: 46,190 and Andrew G. Maher, Ph.D., Reg. No.: 79,399 ("the Representatives"), thank Examiner Weidner for the courtesy of a telephone interview conducted with the Representatives on June 30, 2021. Also participating in the telephone interview was David C. Duffy, Ph.D. Dr. Duffy is the Chief Technology Officer of the Applicant, Quanterix Corporation ("Quanterix"). Before the interview, Representative Pomianek emailed an Agenda to Examiner Weidner, which is attached hereto with supporting materials described below as Appendix 1. The Agenda included (a) proposed claim amendments for discussion (attached as Appendix A to Appendix 1); (b) a copy of Ding, et al. (2021), "Ultrasensitive assays for detection of plasma tau and phosphorylated tau 181 in Alzheimer's disease: a systematic review and meta-analysis," *Translational neurodegeneration*, 10(1), 1-14 ("Ding" attached as Appendix B to Appendix 1); and (c) a copy of Teunissen, et al. (2020), "Plasma p-tau217: from 'new kid' to most promising candidate for Alzheimer's disease blood test," *Brain*, 143(11), 3170-3172 ("Teunissen"; attached as Appendix C to Appendix 1).

During the interview, the Representatives, Examiner Weidner, and Dr. Duffy discussed the rejection of independent claim 92 under 35 U.S.C. 103 over US20100075407 ("Duffy '407") in view of Quanterix 2010 IQT Technology Focus Day ("Quanterix 2010a"). Dr. Duffy is the first named inventor of Duffy '407 and was the presenter of the Quanterix 2010a slides. The Representatives described the context of the claimed method and the frame of mind of a person of ordinary skill in the art at and around the time of the invention and at the time of the earliest

effective filing date (April 11, 2011). The Representatives explained that claim 92, which is directed to preparing a sample from which to measure a level of tau that is derived from a bodily fluid comprising blood (or a blood component selected from plasma and serum) that was contraindicated, as samples derived from cerebrospinal fluid (CSF) were conventionally believed to be required, and that recites limitations related to an analytically quantified amount of tau (less than about 5 pg/mL) and a level of quantification (less than about 0.2 pg/mL) that a person of ordinary skill at that time would not have believed to be necessary, useful or appropriate for patients suspected of having a neurological condition (e.g., as a successful tool for treatment/diagnosis). The Representatives noted while there were a handful of experimental attempts in the prior art purporting to measure tau in blood (at higher levels generally lacking reliability and with high control levels making it questioning whether, in at least the controls, tau was actually being measured), the publication of the assays and data disclosed in Applicant's Application resulted in a clear line of demarcation and sea-change in the field that effectively created or contributed to creating the reasonable expectation of success that was absent at the time Applicant's invention was made and first filed for patent protection.

Dr. Duffy further described the nature and extent of the sea-change in the neurobiomarker industry following the publication of the subject matter of the present Application in 2012, providing specific examples – a development with now heightened significance in view of the recent, widely publicized, FDA approval of Biogen's Alzheimer's drug Aduhelm™, highlighting the importance of considering rapid, easy-to-administer, and accurate blood tests for neurological biomarkers as part of a patient treatment plan this and future novel therapeutics. Dr. Duffy further supported his points by referencing Teunissen, one example of recent commentary on the promise of plasma tau assays for Alzheimer's disease blood tests, as underscoring the dramatic rise in importance of tau blood tests following this sea-change. Dr. Duffy explained that conventional wisdom at the time was that tau was not reliably present in blood, either generally due to the blood-brain barrier or at least at levels that were consistent and meaningfully correlated to any neurological disorder. Furthermore, to the extent the skilled person had attempted to measure tau in blood or was aware of such attempts, conventional ELISA assays useful for measurement of tau in CSF were believed to be sufficient—which was reinforced by the relatively high, possibly

artifactual measurements published from early studies attempting to measure tau in blood using such conventional ELISA assays. Dr. Duffy described the few examples of which he was aware of investigators trying to measure tau in blood using conventional ELISA approaches. As an example, Dr. Duffy pointed to the very large error bars and signal response buried in noise in Mortberg "Assessment of the Cerebral Ischemic/Reperfusion Injury after Cardiac Arrest" *Acta Universitatis Upsaliensis Uppsala*, 2010 ("Mortberg"), and how it was evident to him why the field did not view such data as sufficiently reliable or repeatable to be adopted by the field or drive investigations to deviate from measurement of tau in CSF using industry accepted ELISA techniques.

By contrast, to illustrate the impact on the field of the results disclosed in the present Application, Dr. Duffy referred to Ding, which is a recent meta-analysis review article on ultrasensitive plasma tau assays. Despite multiple ultrasensitive measurement techniques being known for years prior to the filing of the present Application, the timeline evident in Ding (see Table 1 and Table 2) shows that it was not until abruptly after the publication of the subject matter of the present Application in 2012 ("post publication period") that, a vast majority of studies (by numerous different research groups) began measuring tau in blood with high sensitivity assays. Moreover, not surprisingly, most of the earliest post publication studies were performed using the Simoa® method described in various embodiments and working examples in the present Application. Dr. Duffy noted that the dramatic rise in publications with clinically relevant measurements of tau in blood only after publication of the techniques and results disclosed in this Application, and the high prevalence of use of Simoa® assays in Ding is clear and objective support for the contention that industry did not "catch on" (i.e., have a reasonable expectation of success of measuring tau in blood with sufficient accuracy and reproducibility to be useful as an indicator of neurological function or injury) until, and in response to, the teaching of the present Application.

The Representatives emphasized that Ding supports the legal conclusion of non-obviousness of the claimed method. Along these lines, the Representatives noted that, following the breaking of the dam by the invention disclosed in the present Application, competitors have begun filing patent applications directed to ultrasensitive measurement of tau and phosphorylated tau in blood using commercial embodiments of Quanterix's Simoa® tau assay technology by collaborating with Quanterix customers, some of which the Examiner is presently examining.

Dr. Duffy then discussed how the measurements of tau in blood on slide 22 of Quanterix 2010a cited in the Office Action are actually in line with the above description of the state of the art at the time. Significantly, the early-stage experimental results depicted in the slide were produced using a conventional ELISA comparable to that used in Mortberg rather than any ultrasensitive technique such as Simoa®. Dr. Duffy explained that the ~60-80 pg/mL levels of tau in age matched disease free individuals depicted on slide 22 were subsequently determined by the inventors of this Application to be inaccurate and likely the result of non-specific binding artifact, with true tau levels in such samples (measured by the techniques as reported in the present Application) being in some cases in excess of an order magnitude lower. Indeed, Dr. Duffy indicated that for at least the disease-free patients in slide 22, the conventional ELISA was likely measuring background noise rather than true signal indicative of analytically quantifiable tau levels in blood, and that other measurements in the prior art (e.g., Mortberg and Wunderlich et al. *Clinical Neurology and Neurosurgery* 108 (2006) 558-563 ("Wunderlich")) that similarly showed much high levels of tau in blood than in claim 92 may reflect the same artifact.

Examiner Weidner, while noting that further search and/or consideration would be needed, indicated that the explanation of the context of the claimed invention, and the Ding reference in particular, demonstrated potentially compelling secondary considerations consistent with non-obviousness, such that the obviousness rejection in the Office Action may not be supportable and will be reconsidered.

Finally, the Representatives and Examiner Weidner discussed proposed new claim 105, which recites specific steps of a preferred embodiment of the quantification step of claim 92 involving suspending a plurality of capture objects that each include a binding surface having affinity for tau protein molecules in the diluted volume of bodily fluid and then interrogating at least some of those capture objects. The Representatives noted neither Duffy '407 nor Quanterix 2010a disclosed or suggested the combination of steps recited in claim 105, which can provide improvements in the performance of high sensitivity assays (e.g., those of Duffy '407) when measuring the tau in blood/blood-derived samples.

Further remarks responsive to the bases of the rejections are presented immediately below

Rejections Under 35 U.S.C §103

Claims 11, 20-24, and 92-102 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over U.S. Patent Application Publication 2010-0075407 ("Duffy '407") in view of Quanterix 2010a IQT Technology Focus Day ("Quanterix 2010a"; cited on IDS 1/13/20 p. 16).

As discussed during the interview, it would not have been obvious to a person of ordinary skill at the time of the invention and earliest effective filing date of the Application to have prepared a sample obtained from patient suspected of having a neurological condition containing an analytically quantified amount of endogenous tau protein from blood (or a component of blood) of less than about 5 pg/mL using an analytical protein concentration measurement assay having a level of quantification of less than about 0.2 pg/mL, as claimed in independent claim 92. The non-obviousness of this combination of features is supported at least by the fact that a person of ordinary skill at the time would have lacked a reasonable expectation of success for reliably producing a clinically useful analytically quantified sample from blood possessing the tau levels claimed measured at the level of sensitivity claimed, for reasons articulated above in the Interview Summary.

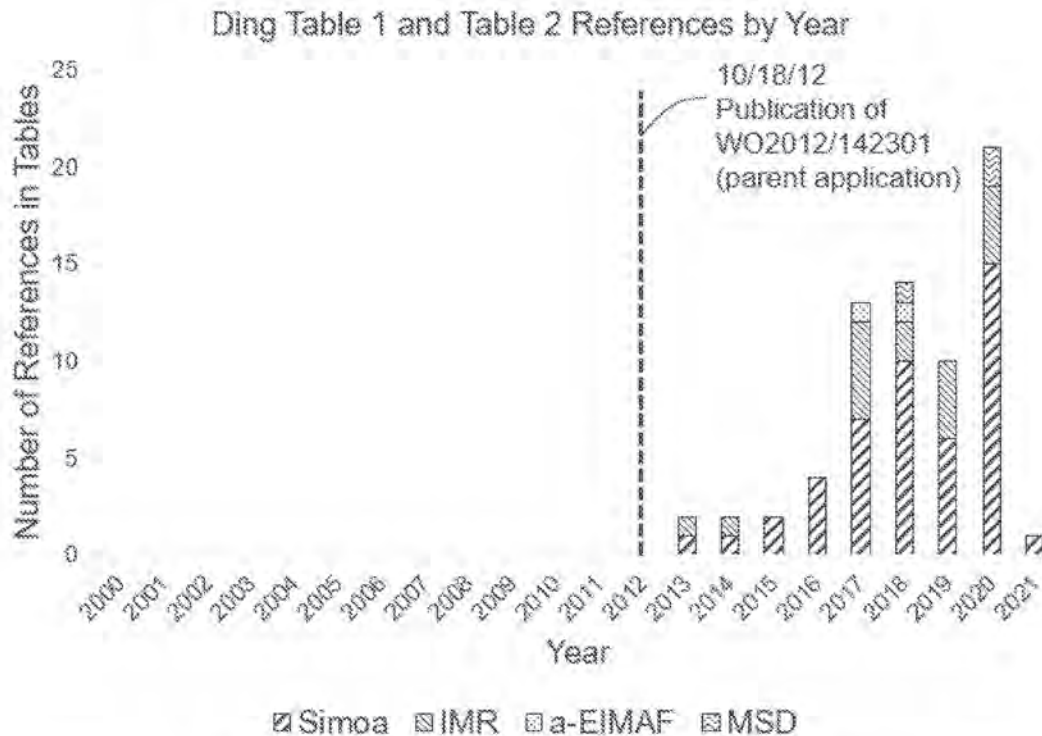
As explained by Dr. Duffy during the interview, before the earliest effective filing date of this Application, there were some attempts at quantifying tau in blood, but with high baseline values and/or lack of reproducibility or clear differentiation of levels in test versus control samples, which at best created doubt as to a reasonable expectation of success in using such measurements as a research or diagnostic tool, and provided no indication of a need if attempting to quantify tau in blood samples to measure at the levels and sensitivities recited in the method as claimed. There was skepticism and doubt as to whether such a measurement would be useful for patients suspected of having a neurological condition and no reason for believing that high-sensitivity assays providing the claimed limit of quantification was needed or advantageous. In Öst et al. (2006), "Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury," *Neurology*, 67(9), 1600-1604 ("Öst"; attached as Appendix 2), the investigators, using conventional ELISA, found a correlation of total tau in CSF (at levels of hundreds to thousands of pg/ml of sample) to patient outcome following traumatic brain injury, but explicitly stated that "[t]otal tau was not detected in serum throughout the study." Öst at Abstract, emphasis added. In Liliang et al., (2010), "τ proteins

in serum predict outcome after severe traumatic brain injury." *Journal of Surgical Research*, 160(2), 302-307 ("Liliang"; attached as Appendix 3), the investigators reported that their measurements of tau protein levels in serum collected from patients following traumatic brain injury having poor outcomes (436.2 ± 473.6 pg/mL) and good outcomes (51.6 ± 81.5 pg/mL) "suggest that in addition to GCS, serum τ protein levels may serve as indicators for the prediction of outcome following serve TBI. However, it should be viewed with caution because of the small sample size and wide standard deviations." Liliang at p. 302; emphasis added. In Wunderlich (published in 2006), a conventional ELISA is used to measure tau protein in serum, and the measured values of tau are all at least 30 pg/mL with only a handful of measurements from acute stroke patients exceeding the 60 pg/mL detection limit of the assay. Wunderlich at p. 560 right column and Fig. 1. In Mortberg, measurements of tau levels in plasma from patients following cardiac arrest using conventional Luminex colorimetric assays show tau values for both good outcome and bad outcome patients approaching and exceeding 50 pg/mL with wide error bars as indicated by the box and whisker plots in Figure 18. Mortberg at p. 48. And in Quanterix 2010a, as discussed during the interview, measurement of total tau in blood using a conventional ELISA (not a Simoa® assay) showed levels of ~60-80 pg/mL even in age-matched disease free patients. Quanterix 2010a at slide 22. As discussed during the interview, Dr. Duffy believes the results in slide 22 of Quanterix 2010a had an artificially inflated baseline value due to background signal calibration mismatch when measured values are near LOD, which would lead to non-AD patients showing values of ~60-80 pg/mL when in fact true values, as were subsequently determined via the presently claimed assay, are typically 0.1-1 pg/mL in serum and 1-10 pg/mL in plasma.

As explained by Dr. Duffy, the Quanterix 2010a slide 22 result is believed to be a common artifact that may also be reflected in the similar results/levels published by other investigators in the references described above. As Dr. Duffy noted during the interview, these publications showing either no tau in blood or widely varying values either buried in noise/error bars or at levels far higher than claimed collectively demonstrate that the conventional wisdom was that measurement of tau in blood was not useful or sufficiently reliable for research/clinical purposes, or at least there was substantial skepticism (i.e., a lack of a reasonable expectation of success) prior to publication of the results reported in this Application. As discussed during the interview, the claimed invention

upon publication and commercial dissemination is believed to have created a reasonable expectation of success resulting in the paradigm shift in the field of tau assays away from CSF to blood/serum/plasma as a medium and towards a new understanding of what are “normal” and symptomatic levels of circulating tau in such samples.

Despite published reports purportedly quantifying tau in blood (examples of which are discussed above), and the general existence of multiple highly sensitive assay techniques being known for years prior to the effective filing date of this Application (more than a decade), the state of knowledge and reasonable expectation of skilled artisans can be inferred by the lack of reports demonstrating analytical quantification of tau in blood at the levels and sensitivity claimed here before this application published. The Ding meta-analysis and review article discussed during the interview as summarized above illustrates this dramatically. The meta-analysis included reports of measurements of plasma tau using the highly sensitive techniques of Simoa®, ImmunoMagnetic Reduction (IMR, around since at least 2006 according to reference 14 in Ding), enhanced immunoassay using multi-arrayed fiber optics conjugated with rolling circle amplification (a-EIMAF), and Meso Scale Discovery (MSD, around since at least 2000 according to referenced 16 in Ding). Tables 1 and 2 in Ding show plasma tau and p-tau reports from various research groups. Notably, and as discussed during the interview, a vast majority of the reports in Tables 1 and 2 of Ding use Simoa® techniques described in the present Application, and all reports came after the publication of International Publication WO2012/142301 on October 18, 2012 from International Application No. PCT/US2012/033343, from which the present Application claims priority. The figure below illustrates the timeline using the frequency of publication dates of the references used in Tables 1 and 2 in Ding starting at the year 2000 (the year of the publication of MSD). Publication of the claimed invention represents a clear line of demarcation in the field evidencing that it was the subject matter of this Application that established a reasonable expectation of success.



As discussed during the interview, tau blood assays at the level of quantification and producing samples with quantified tau at the claimed levels have achieved a marked rise in prominence in the medical community since the publication of the subject matter of the present Application, with the Teunissen reference as one example praising ultrasensitive tau measurement in blood as a tool for Alzheimer's diagnosis. Another example of media reports praising tau measurement blood and acknowledging the notoriety of Quanterix's commercial embodiments of the claimed invention is in the April 19, 2021 article Cairns, E. "A new market beckons for Alzheimer's blood tests" *Evaluate Advantage*, 2021 ("Cairns"; attached as Appendix 4). This article notes that Quanterix's product is comparable in correlation for Alzheimer's pathology to CSF and PET scan testing that require either a spinal tap or lengthy imaging procedures, and quotes Quanterix CEO Kevin Hrusovsky as saying that "the test is 'selling off the charts' at the moment." Cairns at p. 2. The praise for ultrasensitive tau measurements in blood in the medical and the sales

success of the commercial embodiment of the claimed invention both underscore the non-obviousness of claim 92.

For at least the reasons outlined above, the combination of Duffy '407 and Quanterix 2010a does not render claim 92 obvious, and independent claim 92 is patentable over the combination of Duffy '407 and Quanterix 2010a.¹ The remaining claims that stand rejected on this ground depend from claim 92, and are therefore also patentable over Duffy '407 and Quanterix 2010a for at least the reasons outlined above. In addition, it is not conceded that Duffy '407 or Quanterix 2010a, alone or in combination, teach or render obvious any of the features recited in claims 11, 20-24, and 93-102, as asserted by the Patent Office. It is also not conceded that it would have been obvious to combine Duffy '407 and Quanterix 2010a in the manner suggested by the Patent Office.

Claims 11, 20-24, and 92-103 are rejected under pre-A IA 35 U.S.C. 103(a) as being unpatentable over Duffy '407 in view of Quanterix 2010a and further in view of Wunderlich.

Wunderlich fails to cure the deficiencies of Duffy '407 and Quanterix 2010a noted above with respect to independent claim 92. Accordingly, claim 92 and its dependents are also patentable over Duffy '407, Quanterix 2010a, and Wunderlich. In addition, it is not conceded that Wunderlich teaches the combination of features recited in claims 11, 20-24, and 92-103, and it is not conceded that it would have been obvious to combine Duffy '407, Quanterix 2010a, and Wunderlich in the manner suggested in the Office Action.

Withdrawal of the rejection is respectfully requested.

Claims 11, 20-24, and 92-102 are rejected under pre-A IA 35 U.S.C. 103(a) as being unpatentable over Duffy in view of Quanterix 2010a and further in view of Mortberg.

Mortberg fails to cure the deficiencies of Duffy '407 and Quanterix 2010a noted above with respect to independent claim 92. Accordingly, claim 92 and its dependents are also patentable over Duffy '407, Quanterix 2010a, and Mortberg. In addition, it is not conceded that Mortberg teaches the combination of features recited in claims 11, 20-24, and 92-103, and it is not conceded that it

¹ It is also not conceded that Quanterix 2010a, which is a slideshow presented to a closed group of InQTel members is a "printed publication" qualifying it as prior art with respect to the claimed invention.

would have been obvious to combine Duffy '407, Quanterix 2010a, and Mortberg in the manner suggested in the Office Action.

Withdrawal of the rejection is respectfully requested.

Double-Patenting

Claims 11, 20-24, and 92-103 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 11, 24-30, 100-102, 104, 107-114, 132-133, and 135 of copending Application No. 14/111,331 (reference application) in view of Quanterix 2010a.

Without acceding to the correctness of the rejection, Applicant is filing a terminal disclaimer.

Withdrawal of the rejection is respectfully requested.

Claim 11, 20-24, and 92-103 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-25 of U.S. Patent No. 10,393,759.

Without acceding to the correctness of the rejection, Applicant is filing a terminal disclaimer.

Withdrawal of the rejection is respectfully requested.

New Claims

New dependent claims 104-106 have been added. Claims 104-106 patentably distinguish the cited references relied of record at least because of their dependency on independent claim 92 whose patentability has been demonstrated above. Claim 105 recites steps of i. suspending a plurality of capture objects that each include a binding surface having affinity for tau protein molecules in the diluted volume of bodily fluid, ii. immobilizing at least a portion of the tau protein molecules with respect to at least some of the plurality of capture objects suspended in the diluted volume of bodily fluid such that at least some of the capture objects associate with at least one tau protein molecule from the volume of bodily fluid, iii. interrogating at least some of the capture objects associated with at least one tau molecule from the volume of bodily fluid; and iv. determining a measure of tau protein molecule concentration in the volume of bodily fluid based at

least in part on the interrogating step performed in step iii. Neither Duffy '407 nor Quanterix 2010a teach, at least, this combination of features.

As discussed during the interview, suspending capture objects in the diluted volume of bodily fluid can allow for improved analyte-binding efficiency by distributing the beads throughout the volume bodily fluid to increase the rate of freely diffusing analytes encountering the capture objects (e.g., as compared to diffusion to fixed locations on planar substrate surfaces). The claimed steps also can provide the benefit of facilitating collection of the capture objects suspended in the bodily fluid (e.g. during magnetic-assisted processing) for more effective handling and, in some instances, partitioning.

Further, determining a measure of tau protein molecule concentration at least in part by interrogating the same capture objects that were suspended in the volume of bodily fluid, as recited in new claim 105, stands in contrast to the methods disclosed in Duffy '407 and Quanterix 2010a. The cited portions of Duffy '407 are directed to immobilizing a plurality of analyte molecules or particles (e.g., to a first substrate) to form a plurality of complexes, releasing at least a portion of some of the plurality of complexes, determining at least a portion of the plurality of complexes released, and determining a measure of the concentration of the analyte molecules or particles in a fluid sample. Quanterix 2010a at slide 13 shows a similar process (e.g., enzyme release followed by enzyme capture). Such a releasing/dissociation step in Duffy '407 and Quanterix 2010a therefore requires that any objects interrogated (e.g., the second substrate) to determine at least a portion of the plurality of complexes released are not the same objects exposed to an analyte-containing solution and immobilized with analyte molecules or particles. For at least these reasons, claim 105 further distinguishes Duffy '407 and Quanterix 2010a.

General Comments on Dependent Claims

Each of the dependent claims depends from a base claim believed to be in condition for allowance, and Applicant believes that it is unnecessary at this time to argue the allowability of each of the dependent claims individually. Applicant does not, however, ratify, adopt, or concede the correctness of the interpretation of the dependent claims as set forth in the Office Action, nor does Applicant concur that the basis for the rejection of any of the dependent claims is proper.

Therefore, Applicant reserves the right to specifically address the patentability of the dependent claims, if deemed necessary.

CONCLUSION

A Notice of Allowance is respectfully requested. The Examiner is requested to call the undersigned at the telephone number listed below if this communication does not place the case in condition for allowance.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant requests any necessary extension of time. If there is a fee occasioned by this response, including an extension fee, the Director is authorized to charge any deficiency or credit any overpayment in the fees filed, asserted to be filed or which should have been filed herewith to our Deposit Account No. 23/2825, under Docket No. Q0052.70018US03.

Dated: July 16, 2021

Respectfully submitted,

By: /Michael J. Pomianek/
Michael J. Pomianek
Registration No.: 46,190
Andrew G. Maher, PhD
Registration No.: 79,399
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

APPENDIX 1

Nadeau, Patricia

From: Pomianek, Michael
Sent: Tuesday, June 29, 2021 12:29 PM
To: Weidner, Adam
Cc: Maher, Andrew G.
Subject: RE: Interview Request for US Application Number 16522237
Attachments: Proposed Interview Agenda for USSN 16_522237.PDF

Dear Examiner Weidner,

To assist your preparation for the interview tomorrow, I have attached a proposed interview agenda that includes proposed claim amendments and copies of two references we anticipate discussing as appendices.

Thank you again and we look forward to our discussion.

Best regards,
Michael Pomianek
Reg No. 46,190



Michael J. Pomianek, PhD

Pronouns: he/him/his

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This e-mail message and any attachments may contain confidential or privileged information. If you are not the intended recipient, please notify me immediately by replying to this message. Please destroy all copies of this message and any attachments. Thank you.

From: Weidner, Adam <Adam.Weidner@USPTO.GOV>
Sent: Wednesday, June 23, 2021 9:47 AM
To: Pomianek, Michael <Michael.Pomianek@WolfGreenfield.com>
Subject: Interview Request for US Application Number 16522237

The requested telephonic interview for 6-30-21 at 2:30PM EST is granted. If there are any materials you would like considered before the interview, please email or fax those materials at least 24 hours prior to the interview time. You may call me at 571-272-3045 at the interview time or if you have any additional questions.

Thank you.

Docket No.: Q0052 70018US03
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: David Wilson
Application No.: 16/522,237
Confirmation No.: 4736
Filed: July 25, 2019
For: METHODS OF DETERMINING A TREATMENT PROTOCOL FOR
AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A
BRAIN INJURY
Examiner: A. M. Weidner
Art Unit: 1649

PROPOSED INTERVIEW AGENDA

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Examiner Weidner:

Thank you for agreeing to discuss the above-referenced application with us via telephone conference to be held on Wednesday, June 30, 2021 at 2:30 PM (EDT).

The undersigned will be joined by Representative Andrew G. Maher (Applicant's representative; Reg. No. 79,399) and Dr. David C. Duffy (Chief Technology Officer of the Applicant, Quanterix Corporation). Dr. Duffy is authorized to make binding statements on behalf the Applicant.

During the interview, we plan to discuss the rejection of claim 92 under 35 U.S.C. 103 over US20100075407 ("Duffy '407") in view of Quanterix 2010 IQT Technology Focus Day ("Quanterix 2010a"). Dr. Duffy is the first named inventor of Duffy '407 and was the presenter of the Quanterix 2010a slides. As part of the discussion, we would like to discuss potential amendments to the claims, attached as Appendix A. The claims in Appendix A include new dependent claim 105, which recites further details of the analytical protein concentration measurement assay used in step (C) and which further distinguishes the cited references of record

We anticipate discussing the success and recognized importance of the claimed method, especially in view of the growing market for blood-based analytical tau assays for patients suspected of having neurological conditions. In this context we plan on referring to a recent meta-analysis review article on ultrasensitive plasma tau assays by Ding et al. (Ding, et al. (2021). "Ultrasensitive assays for detection of plasma tau and phosphorylated tau 181 in Alzheimer's disease: a systematic review and meta-analysis." *Translational neurodegeneration*, 10(1), 1-14; attached as Appendix B) and a recent commentary on the promise of plasma tau assays for Alzheimer's disease blood tests by Teunissen et al. (Teunissen, et al. (2020). "Plasma p-tau217: from 'new kid' to most promising candidate for Alzheimer's disease blood test." *Brain*, 143(11), 3170-3172; attached as Appendix C).

Additionally, we plan on discussing the nature, circumstances, and content of the Quanterix 2010a reference, including slide 22. We expect the discussion will also include what a person of ordinary skill in the art at the time would have understood and reasonably expected regarding analytical quantification of tau in blood based on Quanterix 2010a and other references cited in the record.

We look forward to our interview.

Dated: June 29, 2021

Respectfully submitted,

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APPENDIX A: Claim Amendments for Discussion During Interview

1 - 10. (Canceled)

11. (Previously Presented) The method of claim 92, wherein the brain injury results from a hypoxic event.

12. - 19. (Canceled)

20. (Previously Presented) The method claim 92, wherein the limit of detection for the analytical protein concentration measurement assay is less than about 0.02 pg/mL.

21. (Previously Presented) The method of claim 92, wherein the brain injury comprises a hypoxic event caused by cardiac arrest.

22. (Previously Presented) The method of claim 92, wherein the brain injury comprises a hypoxic event caused by stroke.

23. (Previously Presented) The method of claim 92, wherein the brain injury comprises a hypoxic event caused by an ischemic event.

24. (Previously Presented) The method of claim 92, wherein the brain injury comprises a hypoxic event caused by a thrombosis.

25. - 91. (Cancelled)

92. (Currently Amended) A method of producing a body fluid sample of a patient ~~recovering from a brain injury~~ suspected of having a neurological condition containing an analytically quantified amount of endogenous tau protein, said method comprising:

(A) obtaining a volume of body fluid comprising blood, or a blood component selected from plasma and serum,

(B) diluting the volume of body fluid, and

(C) quantifying through the use of an analytical protein concentration measurement assay a concentration of tau protein in the volume of body fluid to produce the body fluid sample containing the analytically quantified amount of endogenous tau protein;

wherein the analytically quantified concentration of endogenous tau protein contained in the body fluid sample is less than about 5 pg/ml, and a limit of quantification of the analytical protein concentration measurement assay used in step (C) for quantifying the concentration of tau protein is less than about 0.2 pg/mL.

93. (Previously Presented) The method of claim 92, wherein the analytical protein concentration measurement assay used in step (C) comprises:

i. exposing a plurality of capture objects that each include a binding surface having affinity for tau protein molecules to the volume of body fluid;

ii. immobilizing at least a portion of the tau protein molecules with respect to the plurality of capture objects such that at least some of the capture objects associate with at least one tau protein molecule and a statistically significant fraction of the capture objects do not associate with any tau protein molecules;

iii. determining a proportion of the plurality of capture objects that associated with a tau protein molecule in step ii; and

iv. determining a measure of tau protein molecule concentration in the volume of body fluid based at least in part on the proportion determined in step iii.

94. (Previously Presented) The method of claim 93, wherein the plurality of capture objects comprises a plurality of beads.

95. (Previously Presented) The method of claim 92, wherein the analytical protein concentration measurement assay used in step (C) comprises spatially segregated tau protein molecules into a plurality of locations, wherein a statistically significant fraction of the locations

contain a single tau protein molecule and a statistically significant fraction of the locations do not contain any tau protein molecules

96. (Previously Presented) The method of claim 95, wherein the plurality of locations comprises a plurality of reaction vessels.

97. (Previously Presented) The method of claim 92, wherein each of the tau protein molecules quantified in step (C) is selected from the group consisting of tau 23 (352, 0N3R), tau 24 (383, 0N4R), tau 37 (381, 1N3R), tau 34 (412, 1N4R), tau 39 (410, 2N3R), and tau 40 (441, 2N4R).

98. (Previously Presented) The method of claim 97, wherein at least a portion of the tau protein molecules are phosphorylated.

99. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid comprises blood.

100. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid comprises plasma.

101. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid comprises serum.

102. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid does not comprise cerebrospinal fluid (CSF).

103. (Currently Amended) The method of claim 104 ~~[[92]]~~, wherein the has been collected from the patient within 12 hours of the brain injury.

104. (New) The method of claim 92, wherein the patient suspected of having a neurological condition is recovering from a brain injury.

105. (New) The method of claim 92, wherein the analytical protein concentration measurement assay used in step (C) comprises:

- i. suspending a plurality of capture objects that each include a binding surface having affinity for tau protein molecules in the diluted volume of body fluid,
- ii. immobilizing at least a portion of the tau protein molecules with respect to at least some of the plurality of capture objects suspended in the diluted volume of body fluid such that at least some of the capture objects associate with at least one tau protein molecule from the volume of the body fluid, and
- iii. interrogating at least some of the capture objects associated with at least one tau molecule from the volume of the body of fluid; and
- iv. determining a measure of tau protein molecule concentration in the volume of body fluid based at least in part on the interrogating step performed in step iii.

APPENDIX B: Ding et al., *Translational Neurodegeneration* (2021)

REVIEW

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Ultrasensitive assays for detection of plasma tau and phosphorylated tau 181 in Alzheimer's disease: a systematic review and meta-analysis

Xulong Ding^{1†}, Shuting Zhang^{2†}, Lijun Jiang³, Lu Wang⁴, Tao Li^{3*} and Peng Lei^{1*}

Abstract

A lack of convenient and reliable biomarkers for diagnosis and prognosis is a common challenge for neurodegenerative diseases such as Alzheimer's disease (AD). Recent advancement in ultrasensitive protein assays has allowed the quantification of tau and phosphorylated tau proteins in peripheral plasma. Here we identified 66 eligible studies reporting quantification of plasma tau and phosphorylated tau 181 (ptau181) using four ultrasensitive methods. Meta-analysis of these studies confirmed that the AD patients had significantly higher plasma tau and ptau181 levels compared with controls, and that the plasma tau and ptau181 could predict AD with high-accuracy area under curve of the Receiver Operating Characteristic. Therefore, plasma tau and plasma ptau181 can be considered as biomarkers for AD diagnosis.

Keywords: Tau, Phosphorylated tau 181, Alzheimer's disease, Simoa, IMR, EIMAF/a-EIMAF, MSD, Plasma biomarker

Background

There is no cure for Alzheimer's disease (AD). The lack of early diagnostic biomarkers for selecting prodromal or early-stage patients is one of the roadblocks in clinical trials. The National Institute on Aging—Alzheimer's Association (NIA-AA) has recently proposed a research framework for AD and specified the importance of amyloid-beta (A β), tau, and neurodegeneration [AT(N)] in the biological definition of AD [1]. Although the A β - or tau-positron emission tomography (PET) has been developed, it is yet to be globally available, making the

NIA-AA research framework challenging to put into practice. Therefore, it is urgent to discover convenient biomarkers with early-diagnostic significance.

Tau is a microtubule-associated protein localized primarily in neurons. It is also a primary component of neurofibrillary tangles (NFTs), a pathological hallmark in AD [2]. The loss of normal functions and the gain of toxic functions of Tau have been linked with the pathogenesis of AD [2–7]. Mounting evidence has suggested that the cerebrospinal fluid (CSF) levels of tau and phosphorylated tau are linearly associated with symptom severity of AD [8–11], suggesting tau as a promising biomarker for early diagnosis and prognostic prediction. However, clinical application of CSF biomarkers has been hindered by high cost, invasiveness, and side effects of lumbar punctures, such as positional headache [12].

The detection of tau in plasma has been limited due to its low abundance until recent technical development of ultrasensitive assays. The plasma tau or phosphorylated

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tau levels in the healthy population and diseased patients have been assessed using different technologies such as Single-molecule Array (Simoa) [13], ImmunoMagnetic Reduction (IMR) [14], enhanced immunoassay using multi-arrayed fiber optics conjugated with rolling circle amplification (a-EIMAF) [15] and Meso Scale Discovery (MSD) [16]. However, the plasma tau or phosphorylated tau levels vary among studies, and there is no cut-off threshold between AD and normal elderly. In this systematic review and meta-analysis, we set out to determine the normal range of plasma tau and phosphorylated tau 181 (ptau181) levels in healthy populations stratified by age and sex, and investigate the cut-off thresholds of plasma tau and ptau181 between AD patients and controls.

Methods

Literature search

Literature search was performed in databases Cochrane Library, MEDLINE, EMBASE, PubMed, Web of Science, and Google Scholar by the date of January 21st, 2021, according to the methodology suggested by the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines [17], using the following terms: Alzheimer disease, AD, dementia, Parkinson disease, PD, traumatic brain injury, TBI, plasma tau, plasma total tau, plasma phosphorylated tau, Simoa IMR, EIMAF, a-EIMAF, and MSD. Papers published in an online-first and ahead-of-print manner were included in the analysis. The protocol of the overarching project has been published (PROSPERO registration No. CRD42020151852).

Study selection

The analysis involved three questions. For Question 1, i.e., the normal range of plasma tau/ptau181 in healthy populations, the following criteria were applied: (1) full-text publications in English; and (2) plasma tau and/or ptau181 levels were measured by Simoa, IMR, EIMAF/a-EIMAF, or MSD. Studies were excluded if: (1) without sufficient data to allow for the extraction of plasma tau/ptau181 levels, or (2) the mean age of cohorts in the studies was < 18 years.

For Question 2, i.e., the differences of plasma tau/ptau181 between AD patients and controls, the following selection criteria were applied: (1) full-text publications in English; (2) plasma tau and/or ptau181 levels were measured by Simoa, IMR, EIMAF/a-EIMAF, or MSD; and (3) AD was diagnosed according to the 2011 core clinical NIA-AA [18] or the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association [19] guidelines. Studies were excluded if: (1) studies without sufficient data to allow for the extraction of

plasma tau/ptau181 levels, (2) the mean age of cohorts in the studies was < 18 years, or (3) patients employed in the studies had other cognitive disorders (e.g., mild cognitive impairment [MCI], vascular dementia, and frontotemporal dementia).

Question 3 is to calculate the diagnostic accuracy of plasma tau/ptau181 for AD. For this, we analyzed all publications selected for Question 2, if the reported plasma tau/ptau181 were used for AD diagnosis in the original publications.

Data extraction and quality assessment

Two authors (XLD and STZ) performed the study assessment independently, and data were reported following the PRISMA statement [17]. Any discrepancy would be discussed with additional reviewers (LJJ and LW). Study quality was assessed using the modified version of the Newcastle-Ottawa Scale (NOS) [20]. A score of up to 8 points was assigned to each study based on the quality of population selection, the comparability between groups, and assessment of exposure. For studies with diagnostic tests, we assessed the quality of selected literature by checking items of the Quality Assessment of Diagnostic Accuracy Studies-2 tool (QUADAS-2) [21].

Statistical analysis and heterogeneity exploration

For the normal range of plasma tau or ptau181, the effect sizes and 95% confidence intervals (CI) were calculated using the Random-effect model, which was used to presume that the true effect size varied among studies [21]. Subgroup analysis was performed according to age and sex ratio. For the diagnostic value of plasma tau or ptau181, the weighted mean difference (WMD) with 95% CI was calculated between AD patients and controls with a random-effects model. To evaluate the diagnostic test accuracy, we used the diagnostic accuracy studies module [22] to calculate main outcome measures, including sensitivity and specificity, and the diagnostic odds ratio (DOR). We then fitted a hierarchical summary receiver operating characteristic (HSROC) model using a bivariate regression approach to visualize the relationship between sensitivity and specificity and calculated the area under curve (AUC). Heterogeneity among studies was assessed using the I^2 index. According to the Cochrane handbook, the heterogeneity was classified as low (I^2 index 0%–40%), moderate (30%–60%), substantial (50%–90%), and considerable (75%–100%) [23, 24]. For heterogeneity exploration, meta-regression analysis was performed to evaluate the potential bias factors, while age, sample size, sex ratio, and region were included as four covariates. Furthermore, the results of meta-analyses were assessed by funnel plots and Egger's test [25]. All calculations were carried out using statistical softwares provided by the Cochrane Collaboration (RevMan 5.1) and Stata14.1

(StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

Results

Study inclusions and quality assessment

The search strategy identified 4230 studies without duplication, and 2999 studies were excluded after abstract screening. Of the left 85 studies with full-text content, 19 studies were excluded as they were not a clinical study or lacked a clinical outcome description. Finally 66 studies were included in this meta-analysis (Fig. 1). Based on the modified NOS criteria [20], 53 studies were classified as high-quality, and 13 studies as medium-quality (Table S1). The revised QUADAS-2 [26] determined that the overall quality of studies included was robust. Most studies ranked as low bias and low applicability concern (Fig. S1).

The characteristics, including number of subjects, average age, male percentage, and plasma tau/ptau181

levels of the 66 studies are summarized in Tables 1 and 2. Forty-one studies using Simoa (15,490 healthy controls), 15 studies using IMR (727 healthy controls), and two studies using a-EIMAF (189 healthy controls) were included for plasma tau studies. Twenty-two studies focused on AD, comprising 1456 patients with AD and 1973 controls. Only studies using Simoa (13 studies, 1189 AD patients and 1611 controls) and IMR (9 studies, 267 AD patients and 362 controls) were included for analysis since there were insufficient data for a-EIMAF. For ptau181, six studies using the Simoa - Karikari method (not the commercial p-tau181 version) (1424 healthy controls) and three using MSD (440 healthy controls) were included for the normal range analysis. Four studies using Simoa (392 AD patients and 773 controls) and three studies using MSD (231 AD patients and 440 controls) were identified for analysis related to AD diagnosis.

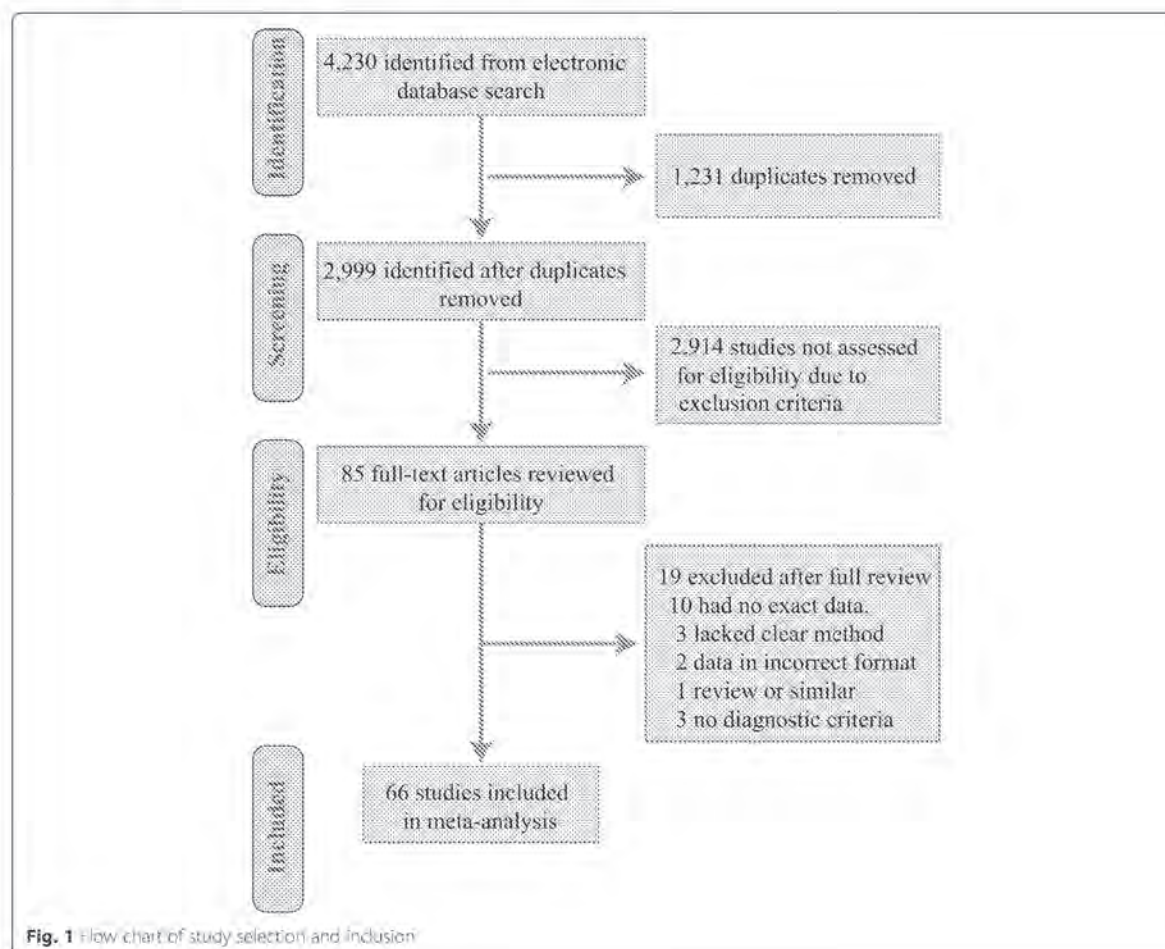


Table 1 Basic characteristics of included studies for plasma tau analysis

Study	Subjects (n)	Male (%)	Age, years (mean $\pm$ SD)	Plasma tau (pg/ml)	Research question	Method
Shanlim et al. (2014) [27]	CN (47)	NA	28 $\pm$ 14.07	4.5 $\pm$ 5.66	1	Simoa
Bogoslovsky et al. (2015) [28]	CN (69)	51	45 $\pm$ 15.5	4.34 $\pm$ 1.77	1	Simoa
Olivera et al. (2015) [29]	CN (28)	96.4	28.40 $\pm$ 4.47	0.63 $\pm$ 0.48	1	Simoa
Dage et al. (2016) [30]	CN (378)	61.4	80 $\pm$ 5.19	4.14 $\pm$ 1.56	1	Simoa
Alosco et al. (2017) [31]	CN (25)	100	55.16 $\pm$ 7.95	2.46 $\pm$ 0.57	1	Simoa
Mielke et al. (2017) [32]	CN (335)	62.4	80.8 $\pm$ 4.8	4.2 $\pm$ 1.5	1	Simoa
Müller et al. (2017) [33]	CN (134)	59.7	68.4 $\pm$ 5	3.6 $\pm$ 1.7	1	Simoa
Kasai et al. (2017) [34]	CN (22)	54.5	37.4 $\pm$ 12.0	0.470 $\pm$ 0.232	1	Simoa
Foiani et al. (2018) [35]	CN (22)	41	68.7 $\pm$ 6.5	1.67 $\pm$ 0.50	1	Simoa
Bergman et al. (2018) [36]	CN (36)	0	30 $\pm$ 4	6.24 $\pm$ 2.76	1	Simoa
Lippa et al. (2018) [37]	CN (42)	90.5	36.21 $\pm$ 11.69	2.81 $\pm$ 1.20	1	Simoa
Verberk et al. (2018) [38]	CN (191)	63	59 $\pm$ 9	3.18 $\pm$ 1.07	1	Simoa
Shanlim et al. (2018) [39]	CN (19)	NA	25.0 $\pm$ 8.89	1.8 $\pm$ 1.48	1	Simoa
Wallace et al. (2018) [40]	CN (13)	100	18.5 $\pm$ 1.7	2.56 $\pm$ 1.02	1	Simoa
Moramedj et al. (2018) [41]	CN (24)	91.7	30.9 $\pm$ 7.77	2.48 $\pm$ 1.94	1	Simoa
Fortea et al. (2018) [42]	CN (67)	30	52.05 $\pm$ 5.50	2.23 $\pm$ 1.63	1	Simoa
Zeitlberger et al. (2018) [43]	CN (13)	46.2	37	2.08 $\pm$ 1.23	1	Simoa
Shi et al. (2019) [44]	CN (87)	41.4	64.77 $\pm$ 7.40	3.56 $\pm$ 1.84	1	Simoa
Kitaguchi et al. (2019) [45]	CN (11)	45.4	67.8 $\pm$ 3.71	0.63 $\pm$ 0.3	1	Simoa
Pase et al. (2019) [46]	CN (3232)	46.8	58 $\pm$ 14	3.93 $\pm$ 1.11	1	Simoa
Korley et al. (2019) [47]	CN (63)	63.5	39.0 $\pm$ 20.7	3.5 $\pm$ 3.19	1	Simoa
Kritikos et al. (2020) [48]	CN (398)	94.72	54.8 $\pm$ 8.1	1.67 $\pm$ 0.685	1	Simoa
Wolf et al. (2020) [49]	CN (4444)	42	71.9 $\pm$ 7.5	2.6 $\pm$ 2.3	1	Simoa
Verberk et al. (2020) [50]	CN (241)	60	61.9 $\pm$ 10	3.1 $\pm$ 1	1	Simoa
Pattinson et al. (2020) [51]	CN (18)	85.3	35.56 $\pm$ 12.39	2.57 $\pm$ 1.01	1	Simoa
Romero et al. (2020) [52]	CN (3472)	46	54.9 $\pm$ 13.2	3.9 $\pm$ 1.11	1	Simoa
Petersen et al. (2020) [53]	CN (225)	51.5	45.7 $\pm$ 7.1	2.4 $\pm$ 1.8	1	Simoa
Cantero et al. (2020) [54]	CN (57)	47.4	67.7 $\pm$ 3.4	3.1 $\pm$ 1.5	1	Simoa
Zetterberg et al. (2013) [55]	CN (25)	24	74 $\pm$ 6.7	4.45 $\pm$ 2.83	1 and 2	Simoa
	AD (54)	31.5	75.1 $\pm$ 6.2	8.80 $\pm$ 10.1		
Mattsson et al. (2016) ADNI [56]	CN (189)	55	75.9 $\pm$ 4.9	2.58 $\pm$ 1.19	1 and 2	Simoa
	AD (179)	52	75.2 $\pm$ 7.4	3.12 $\pm$ 1.50		
Mattsson et al. (2016) BioFINDER [56]	CN (274)	39	72.9 $\pm$ 4.9	5.58 $\pm$ 2.51	1 and 2	Simoa
	AD (61)	42	76.4 $\pm$ 4.7	5.37 $\pm$ 2.56		
Shi et al. (2016) [44]	CN (106)	54.7	67.1 $\pm$ 7.4	2.405 $\pm$ 2.76	1, 2 and 3	Simoa
	AD (106)	53.8	69.5 $\pm$ 8.1	3.870 $\pm$ 2.22		
Kovacs et al. (2017) [57]	CN (18)	50	73.7	1.68 $\pm$ 0.17	1 and 2	Simoa
	AD (21)	23.8	77	7.5 $\pm$ 3.2		
Deters et al. (2017) [58]	CN (166)	57.2	75.2 $\pm$ 5.1	2.71 $\pm$ 1	1 and 2	Simoa
	AD (168)	51.8	75.3 $\pm$ 7.3	3.13 $\pm$ 1.3		
Chen et al. (2017) [59]	CN (151)	58.9	75.7 $\pm$ 4.9	2.7 $\pm$ 1.1	1 and 2	Simoa
	AD (149)	55.7	76.1 $\pm$ 7.3	3.2 $\pm$ 1.3		
Mielke et al. (2018) [60]	CN (172)	69.2	71.9 $\pm$ 9.5	5.9 $\pm$ 1.90	1 and 2	Simoa

Table 1 Basic characteristics of included studies for plasma tau analysis (Continued)

Study	Subjects (n)	Male (%)	Age, years (mean $\pm$ SD)	Plasma tau (pg/ml)	Research question	Method
Park et al. (2019) [61]	AD (40)	23	67.7 $\pm$ 9.2	7.2 $\pm$ 2.80	1, 2 and 3	Simoa
	CN (172)	40.4	71.08 $\pm$ 1.0	2.37 $\pm$ 0.1		
	AD (40)	13.3	75.87 $\pm$ 2.1	3.36 $\pm$ 0.3		
Li et al. (2019) [62]	CN (9)	44.4	61.78 $\pm$ 10.52	4.62 $\pm$ 0.50	1 and 2	Simoa
	AD (53)	42.3	68.39 $\pm$ 9.65	5.47 $\pm$ 2.69		
Startin et al. (2019) [63]	CN (27)	59.3	49.26 $\pm$ 10.40	1.49 $\pm$ 1.26	1 and 2	Simoa
	AD (27)	66.7	59.33 $\pm$ 4.04	1.45 $\pm$ 1.02		
Sugarman et al. (2020) [64]	CN (238)	37.4	72.38 $\pm$ 7.69	3.22 $\pm$ 2.73	1, 2 and 3	Simoa
	AD (156)	55.8	76.74 $\pm$ 8.12	3.73 $\pm$ 3.01		
Fossati et al. (2020) [65]	CN (68)	35.3	67.71 $\pm$ 8.54	2.74 $\pm$ 0.76	1, 2 and 3	Simoa
	AD (29)	34.5	72.81 $\pm$ 9.69	3.67 $\pm$ 1.06		
Deniz et al. (2020) [66]	CN (162)	23.5	82.7 $\pm$ 8.15	3.84 $\pm$ 2.27	1 and 2	Simoa
	AD (159)	31.2	78.2 $\pm$ 8.97	3.75 $\pm$ 2.36		
Lin et al. (2018) [67]	CN (35)	40.0	62.6 $\pm$ 9.7	12.12 $\pm$ 0.96	1	IMR
Chi et al. (2019) [68]	CN (42)	79	59 $\pm$ 11.1	18.2 $\pm$ 13.78	1	IMR
Chen et al. (2019) [69]	CN (13)	69.2	73 $\pm$ 13.0	18.7 $\pm$ 5.63	1	IMR
Chen et al. (2020) [70]	CN (28)	28.6	61.1 $\pm$ 4.9	14.67 $\pm$ 8.49	1	IMR
Fang et al. (2020) [71]	CN-young (43)	67	38.7 $\pm$ 13.5	14.9 $\pm$ 5.5	1	IMR
	CN-old (34)	41	70.3 $\pm$ 5.8	15.0 $\pm$ 7.3		
Chiu et al. (2017) [72]	CN-middle (56)	29.1	58.1 $\pm$ 4.9	14.35 $\pm$ 6.49	1	IMR
	CN-old (70)	45.7	73.6 $\pm$ 6.3	18.14 $\pm$ 7.33		
Chiu et al. (2013) [73]	CN (30)	43.3	64.4 $\pm$ 9.5	15.6 $\pm$ 6.9	1 and 2	IMR
	AD (10)	40	69.3 $\pm$ 9.4	53.9 $\pm$ 11.7		
Ezen et al. (2014) [74]	CN (20)	50	63.7 $\pm$ 7.9	13.5 $\pm$ 5.5	1 and 2	IMR
	AD (14)	28.6	64.9 $\pm$ 11.5	46.7 $\pm$ 2.0		
Yang et al. (2017) [75]	CN (66)	NA	64.6 $\pm$ 8.6	13.37 $\pm$ 7.77	1 and 2	IMR
	AD (29)		72.2 $\pm$ 9.9	55.44 $\pm$ 27.45		
Lee et al. (2017) [76]	CN-young (44)	68.1	38.4 $\pm$ 13.5	14.9 $\pm$ 5.5	1 and 2	IMR
	CN-old (34)	41.2	70.3 $\pm$ 5.8	15.0 $\pm$ 7.3		
	AD (62)	46.8	72.1 $\pm$ 11.1	47.5 $\pm$ 18.9		
Lue et al. (2017) BSHR [77]	CN (16)	25	81.9 $\pm$ 1.5	20.48 $\pm$ 1.24	1 and 2	IMR
	AD (16)	43.8	82.5 $\pm$ 1.4	34.52 $\pm$ 3.75		
Lue et al. (2017) NTUH [77]	CN (61)	39.3	64.2 $\pm$ 1.1	13.98 $\pm$ 1.89	1 and 2	IMR
	AD (31)	54.8	72.5 $\pm$ 1.8	52.47 $\pm$ 2.72		
Yang et al. (2018) [78]	CN (23)	NA	67.5 $\pm$ 7.1	18.85 $\pm$ 10.16	1 and 2	IMR
	AD (21)		78.8 $\pm$ 7.9	37.34 $\pm$ 12.29		
Chiu et al. (2019) BSHR [79]	CN (16)	NA	81.9 $\pm$ 6	20.48 $\pm$ 4.96	1 and 2	IMR
	AD (16)		82.5 $\pm$ 1.4	34.52 $\pm$ 1.4		
Chiu et al. (2019) NTUH [79]	CN (37)	NA	66.1 $\pm$ 8.3	16.61 $\pm$ 9.18	1 and 2	IMR
	AD (25)		78.1 $\pm$ 7.3	43.35 $\pm$ 15.14		
Biao et al. (2020) [80]	CN (57)	45.6	67.9 $\pm$ 9.5	20.65 $\pm$ 3.52	1 and 2	IMR

Table 1 Basic characteristics of included studies for plasma tau analysis (Continued)

Study	Subjects (n)	Male (%)	Age, years (mean $\pm$ SD)	Plasma tau (pg/ml)	Research question	Method
Liu et al. (2020) [51]	AD (40)	37.5	68.1 $\pm$ 9.0	25.9 $\pm$ 8.12	1 and 2	IMR
	CN (2)	50	37.4 $\pm$ 2.8	17.4 $\pm$ 1.1		
	AD (3)	33.3	71.3 $\pm$ 4.0	28.3 $\pm$ 4.0		
Rubenstein et al. (2017) [82]	CN (20)	76	40.5 $\pm$ 14.2	0.063 $\pm$ 0.014	1	a-EIMAF
Gardner et al. (2018) [83]	CN-young (79)	73.4	25.8 $\pm$ 7.3	0.079 $\pm$ 0.0087	1	a-EIMAF
	CN-middle-aged (60)	66.7	56.0 $\pm$ 5.9	0.078 $\pm$ 0.008		
	CN-older (30)	63.3	68.0 $\pm$ 8.4	0.079 $\pm$ 0.0065		

Plasma tau and ptau181 levels in healthy cohorts

Subgroup analysis was performed according to age and sex ratio, among studies using Simoa, IMR, a-EIMAF, and MSD, respectively. Based on the data retrieved, the average age of cohort stratification was classified as young (< 40 years old), middle-aged (40–60 years old), and old (> 60

years). The sex ratio was defined as low (less than 40% male), middle (40%–60% male), and high (over 60% male).

Among the 66 studies, 58 studies that used Simoa, IMR, or a-EIMAF were included for the analysis of normal range of plasma tau, as the MSD studies focused on CSF tau. Forty-one publications, reporting 42

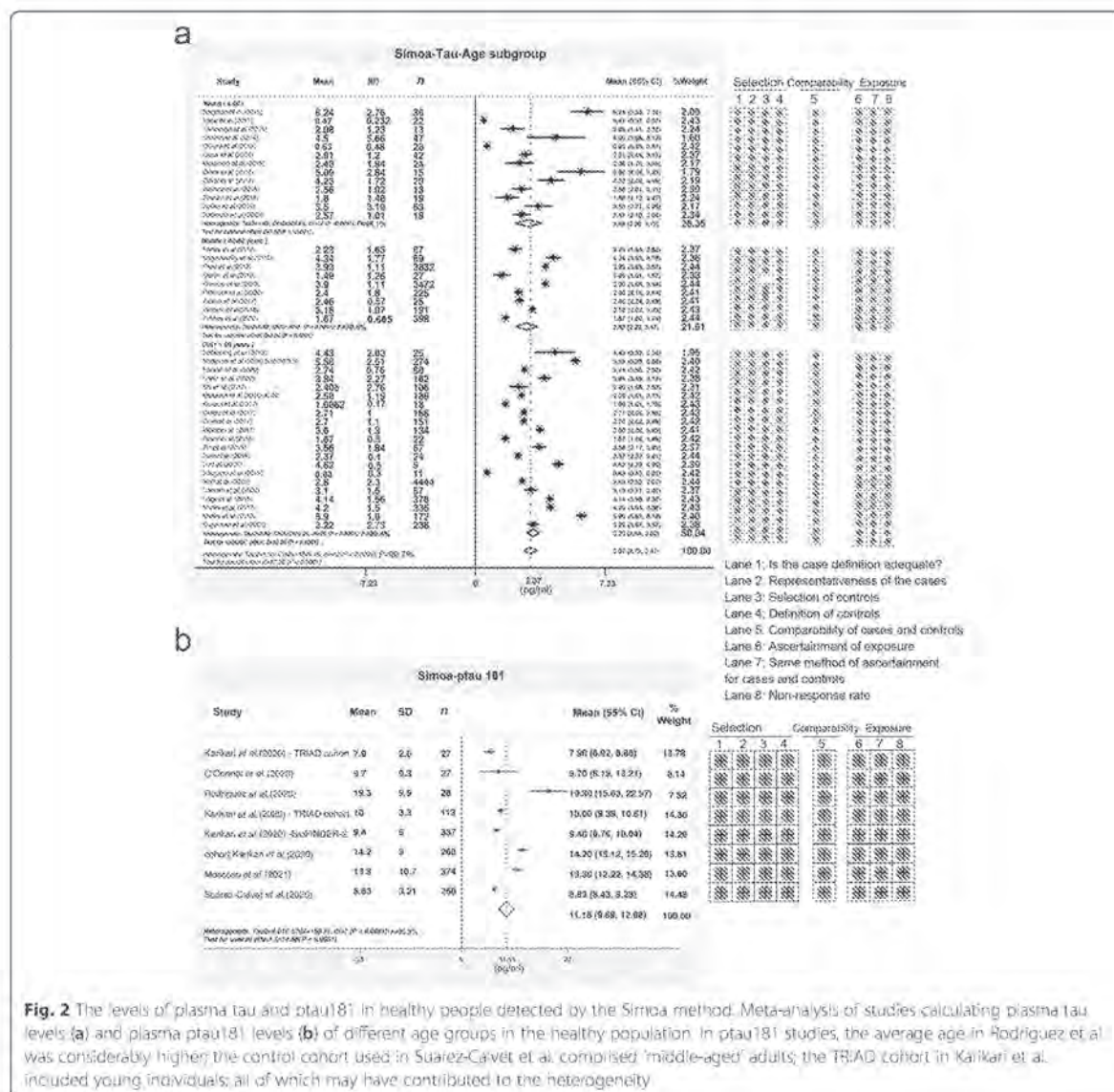
Table 2 Basic characteristics of included studies for plasma ptau181 analysis

Study	Subjects (n)	Male (%)	Age, years (mean $\pm$ SD)	P-tau-181 (pg/ml)	Research question	Method
Suárez-Calvet et al. (2020) [84]	CN (250)	38	60.6 $\pm$ 4.44	8.83 $\pm$ 3.21	1	Simoa - Karikari method
Moscato et al. (2021) [85]	CN (374)	47.1	74.8 $\pm$ 6.6	13.3 $\pm$ 10.7	1	Simoa - Karikari method
O'Connor et al. (2020) [86]	CN (27)	41	58.1 $\pm$ 10.7	9.7 $\pm$ 9.3	1, 2 and 3	Simoa - Karikari method
	AD (19)	63	50.7 $\pm$ 10.0	23.7 $\pm$ 10.5		
Rodríguez et al. (2020) [87]	CN (28)	35.7	82.2 $\pm$ 6.5	19.3 $\pm$ 9.9	1, 2 and 3	Simoa - Karikari method
	AD (77)	46.1	81.7 $\pm$ 7.6	28.4 $\pm$ 9.6		
Karikari et al. (2020) - TRIAD cohort [88]	CN-young (27)	37	22.7 $\pm$ 1.9	7.9 $\pm$ 2.6	1, 2 and 3	Simoa - Karikari method
	CN-old (113)	36	69.2 $\pm$ 9.7	10 $\pm$ 3.3		
	AD (53)	55	64.6 $\pm$ 9.2	24.9 $\pm$ 7.8		
Karikari et al. (2020) - BioFINDER-2 cohort [88]	CN-old (337)	46	63.1 $\pm$ 5.0	9.4 $\pm$ 6.0	1, 2 and 3	Simoa - Karikari method
	AD (126)	47	74.0 $\pm$ 6.9	19.2 $\pm$ 9.4		
Karikari et al. (2020) [45]	CN (268)	51.1	73.5 $\pm$ 6.5	14.2 $\pm$ 9.0	1, 2 and 3	Simoa - Karikari method
	AD (137)	52.2	73.4 $\pm$ 8.2	25.8 $\pm$ 8.6		
Mielke et al. (2018) [60]	CN (172)	69.2	71.9 $\pm$ 9.5	6.4 $\pm$ 6.4	1, 2 and 3	MSD
	AD (40)	23	67.7 $\pm$ 9.2	11.6 $\pm$ 4.1		
Thijssen et al. (2020) [89]	CN (69)	53.6	60.8 $\pm$ 2.2	2.4 $\pm$ 3	1, 2 and 3	MSD
	AD (56)	41.1	65 $\pm$ 9	8.4 $\pm$ 4		
Janežič et al. (2020) - Cohort1 [90]	CN (26)	38.5	74 $\pm$ 5.2	1.3 $\pm$ 1.1	1, 2 and 3	MSD
	AD (38)	44.7	73 $\pm$ 8.1	4.4 $\pm$ 2.3		
Janežič et al. (2020) - Cohort2 [90]	CN (126)	61.9	71 $\pm$ 5.2	1.2 $\pm$ 0.59	1, 2 and 3	MSD
	AD (81)	43.2	73 $\pm$ 5.2	2.8 $\pm$ 2.07		
Janežič et al. (2020) - Cohort3 [90]	CN (47)	59.6	83 $\pm$ 9.6	1.8 $\pm$ 1.04	1, 2 and 3	MSD
	AD (16)	25.0	83 $\pm$ 7.4	4 $\pm$ 2.07		

cohorts, were retrieved using the Simoa technology [8, 27–29, 31–44, 46–49, 51–66, 91–93]. Our results indicated that the average plasma tau level in healthy populations was 3.07 pg/ml (95% CI 2.72–3.41, $I^2 = 99.7\%$, $P < 0.0001$). The plasma tau levels in the young, middle-aged, and old groups were 2.96 pg/ml (95% CI 2.20–3.72), 2.85 pg/ml (95% CI 2.22–3.47), and 3.23 pg/ml (95% CI 2.84–3.62), respectively (Fig. 2a), without significant differences ($P = 0.099$). However, significant differences were identified in the subgroup analysis by sex ratio (4.15 pg/ml vs 2.64 pg/ml vs 3.24 pg/ml; $P < 0.0001$; Fig. S2). Fifteen publications [67–81] reporting 20 cohorts were retrieved using IMR and two publications [82, 83] reporting four cohorts were

retrieved using α -EIMAF. The effect sizes (ESs) for plasma tau levels were 16.30 pg/ml (95% CI 14.61–17.99, $\bar{r}^2 = 92.7\%$, $P < 0.0001$, Fig. S3) and 76.14 fg/ml (95% CI 72.34–79.93, $\bar{r}^2 = 88.8\%$, $P < 0.0001$, Fig. S4), respectively.

Nine studies that used Simoa or MSD methods were included for the analysis of normal range of plasma ptau181. Six publications, reporting seven cohorts, were retrieved using the Simoa technology [45, 84–88], including 1424 healthy subjects. The ES for plasma ptau181 levels in healthy populations was 11.18 pg/ml (95% CI 9.68–12.68, $I^2 = 95.9\%$, $P < 0.0001$, Fig. 2b). There were no significant differences in plasma ptau181 level in the subgroup analysis concerning age ($P = 0.181$).

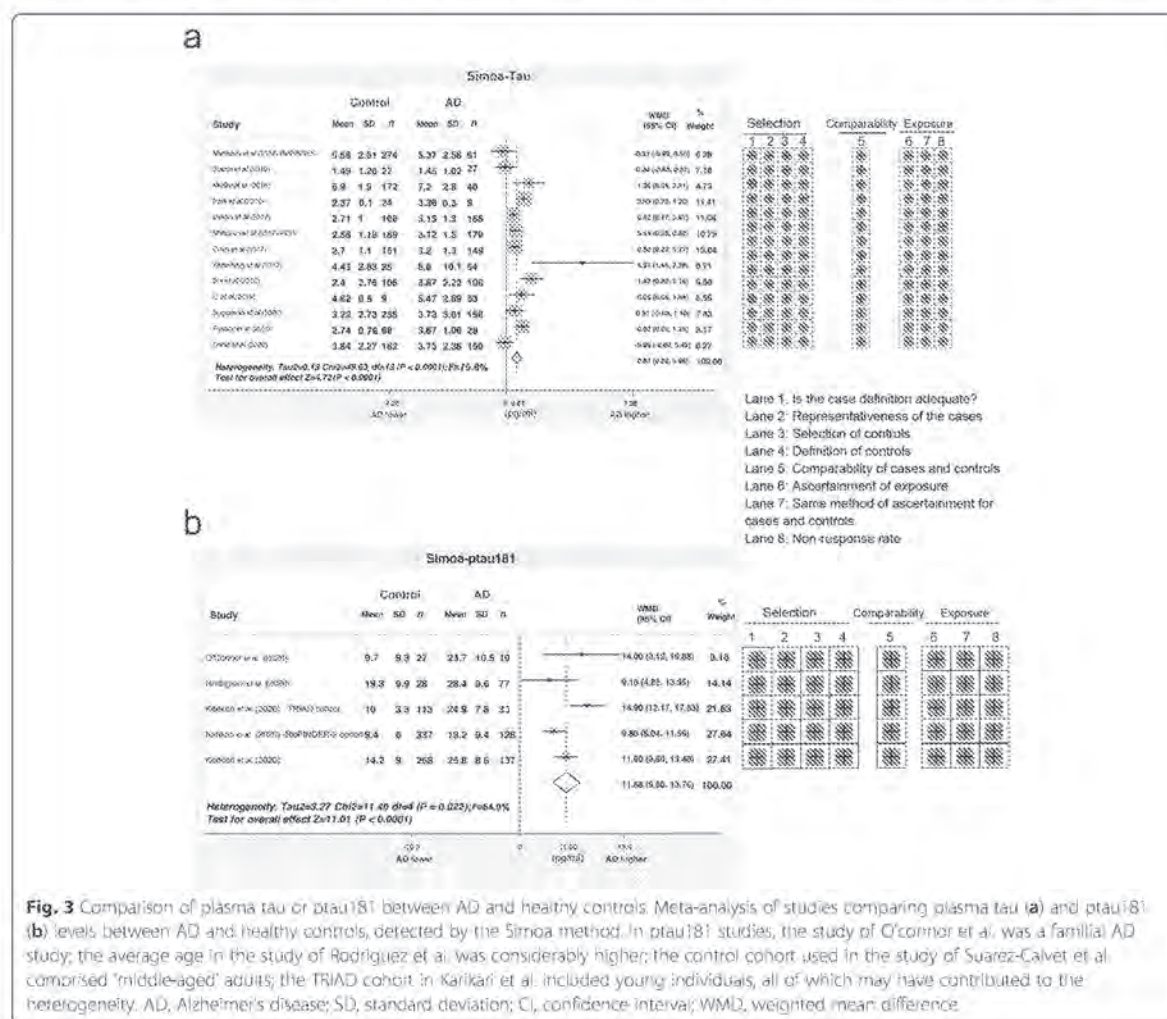


or the sex ratio ($P=0.168$, Fig. S2). Three publications [60, 89, 90] reporting five cohorts using the MSD method, including 440 healthy subjects who could not be grouped by age or sex ratio, were identified. The ES for plasma ptau181 levels measured by MSD was 2.48 pg/ml (95% CI 1.57–3.37, $I^2=97.0\%$, $P<0.0001$; Fig. S4).

Plasma total tau and ptau181 in AD patients and controls

We then compared plasma tau and ptau181 between AD and controls. The random-effects model of meta-analysis with subgroup analysis was performed according to age and sex ratio. Among the 66 studies, 22 studies that used Simoa and IMR were included for the analysis of difference of plasma tau between AD and controls, and the number of studies that used other technologies was insufficient for analysis. For the Simoa method, 13 papers, reporting 14 cohorts [44, 55–66], were retrieved with a total of 1189 AD patients with 1611 controls. Our

analysis revealed a significantly higher plasma tau level in patients with AD, with an average WMD value of 0.61 (95% CI 0.36–0.86, $I^2=75.8\%$, $P<0.0001$; Fig. 3a). The WMDs for subgroups divided by different male compositions were 0.48 (<40% male, 95% CI -0.18–1.14), 0.99 (40%–60% male, 95% CI 0.54–1.44) and 1.30 (>60% male, 95% CI 0.39–2.21), respectively, indicating a strong effect of sex on plasma tau levels in AD patients (Fig. S5). Meta-regression analysis identified that only the sample size significantly contributed to the high heterogeneity ($P=0.027$, Table S2). The funnel plots and the Egger's test suggested no publication bias ($P=0.133$) (Fig. S6). Similarly, 9 papers [72–74, 76–81] reporting 11 cohorts using the IMR method (362 controls and 267 patients) were identified (Fig. S5), showing an average WMD value of 24.83 (95% CI 15.70–33.96, $I^2=98.9\%$, $P<0.0001$). There was no publication bias ($P=0.175$, Fig. S6), but



the year of publication ($P = 0.013$) may influence the heterogeneity (Table S2).

Seven studies that used Simoa and MSD were included for the analysis of the plasma ptau181 difference between AD and controls. Four publications reporting five cohorts [45, 86–88] using the Simoa technology were retrieved, resulting in a total of 773 controls and 392 patients. The plasma ptau181 levels were significantly elevated in AD patients, with an average WMD value of 11.68 (95% CI 9.60–13.76, $I^2 = 64.9\%$, $P < 0.0001$, Fig. 3b). The funnel plots suggested no publication bias for plasma ptau181 ($P = 0.635$, Fig. S6). Similar results were found based on three papers reporting five cohorts [60, 89, 90] using the MSD method (440 controls and 231 patients), with a WMD of 3.53 (95% CI 1.97–5.09, $I^2 = 93.0\%$, $P < 0.0001$, Fig. S5). There was also no publication bias ($P = 0.055$, Fig. S6).

The diagnostic accuracy of plasma tau/ptau181 for AD

Among the 66 studies, four studies reporting five cohorts [44, 61, 64, 65] were pooled for meta-analysis of diagnostic accuracy of plasma tau using the Simoa method. The estimate values of diagnostic accuracy are summarized in Table S3. The pooled sensitivity and specificity of plasma tau to predict AD were 0.75 (95% CI 0.60–0.86) and 0.69 (95% CI 0.57–0.79), respectively (Fig. 4a). The DOR was 6.16 (95% CI 3.02–12.53), and the AUC of the HSROC curve was 0.77 (95% CI 0.74–0.81) (Fig. 4b). Fagan's nomogram showed that the probability of AD increased from 25% (pre-test probability) to 45% (post-test probability) when plasma tau level was above the threshold, while the probability of AD decreased to as low as 11% when plasma tau level was below the threshold (Fig. 4c).

Four studies using the Simoa method reporting five cohorts [45, 86–88] were pooled for meta-analysis to test the diagnostic accuracy of plasma ptau181. The estimate values of diagnostic accuracy are summarized in Table S3. The pooled sensitivity and specificity of ptau181 were 0.89 (95% CI 0.81–0.93) and 0.86 (95% CI 0.79–0.91), respectively (Fig. 4d). The DOR was 46 (95% CI 18–123), and the AUC of the HSROC curve was 0.93 (95% CI 0.91–0.95) (Fig. 4e). The probability of AD increased from 25% (pre-test probability) to 67% (post-test probability) when plasma ptau181 level was above the threshold and decreased to as low as 4% when plasma ptau181 level below the threshold (Fig. 4f). Consistently, two studies using the MSD method reporting four cohorts [89, 90] were pooled for meta-analysis. The estimate values of diagnostic accuracy are summarized in Table S3. The pooled sensitivity of ptau181 was 0.87 (95% CI 0.78–0.92), and the pooled specificity was 0.79 (95% CI 0.73–0.83, Fig. S7). The DOR was 23.98 (95% CI 10.14–56.69), and the AUC of the HSROC curve was 0.86 (95% CI 0.83–0.89, Fig. S7). The

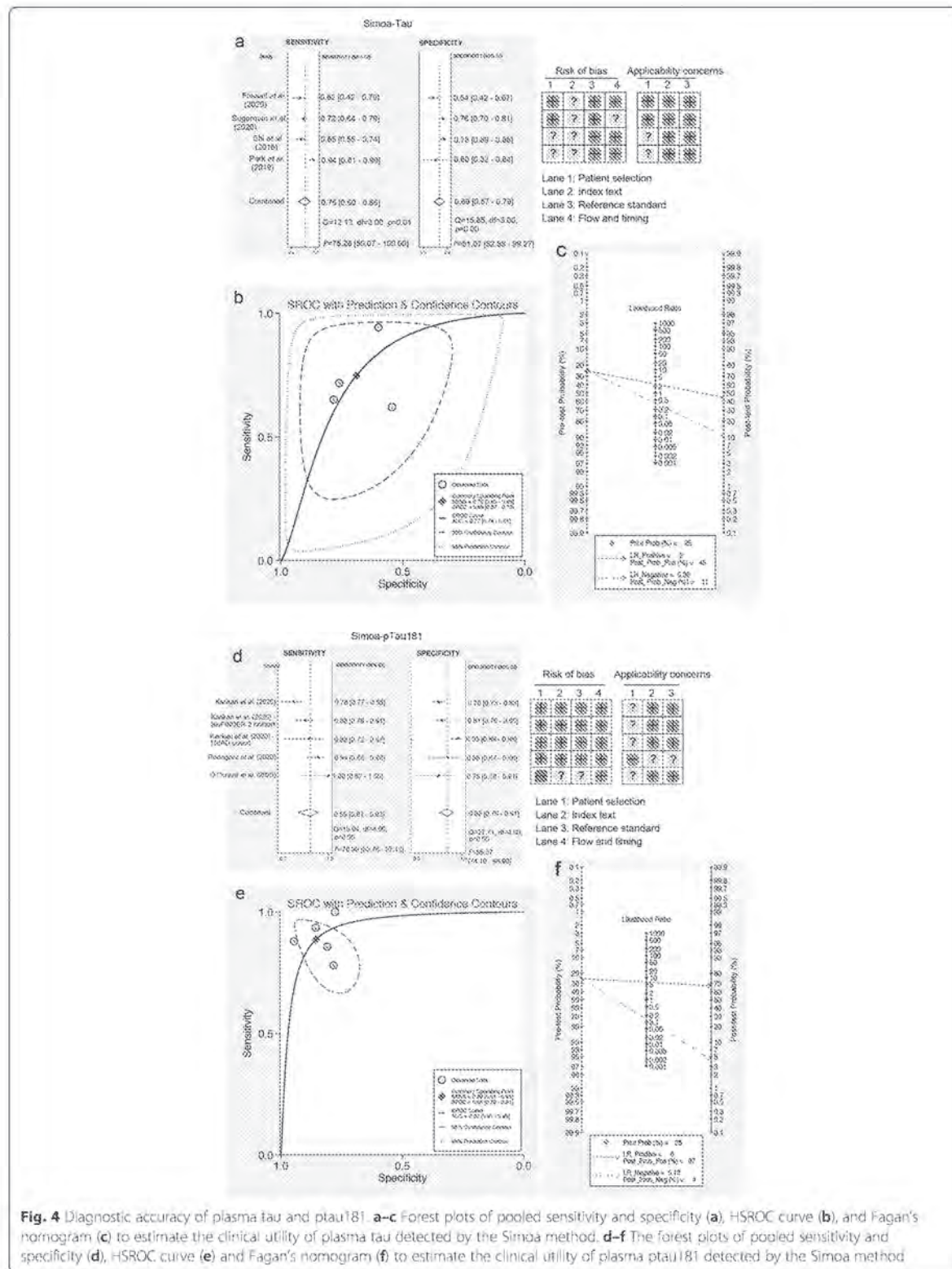
probability of AD increased from 25% (pre-test probability) to 58% (post-test probability) when plasma ptau181 level was above the threshold, and decreased to 5% when plasma ptau181 level was below the threshold (Fig. S7).

Discussion

With a growing interest in plasma tau detection during the last 10 years, heterogeneity between studies has been consistently presented. Besides, given the inconsistency between publications regarding the plasma tau levels in AD (compared to control), it is difficult to determine the suitability of plasma tau/ptau181 to predict AD. In this meta-analysis, we found that both plasma tau and ptau181 have diagnostic values, and both of them are significantly higher in AD patients than in controls. We also established the average plasma tau and ptau181 levels based on the current literature, which may be used as a reference point in future research.

Currently, there are four ultrasensitive assays that can be used for plasma tau and ptau detection. In this meta-analysis, we examined them independently. Despite the differences in absolute values of plasma tau and ptau, results from all assays support the notion that both plasma tau and ptau181 are elevated in AD compared with healthy controls. Based on the available publications of each method, we have been able to calculate the sensitivity and diagnostic accuracy for AD of studies using Simoa (tau AUC: 0.77; ptau181 AUC: 0.93), and MSD methods (ptau181 AUC: 0.86), while the other two methods had limited applications in AD research.

Both A β and tau can now be visualized in the brain using PET and be measured in the CSF. Although medical history and cognitive and neurologic examinations remain the most important diagnostic tool in the clinic [34], these new techniques can assist the diagnosis of AD [1]. However, the associated cost and infrastructure requirements have limited their use, especially in developing countries. It is critical to accurately measure AD-associated proteins in plasma and determine their relationships with brain and CSF contents. Proteins in plasma may reflect protein levels in the brain and CSF, especially in the state of illness. For example, the correlation between CSF and plasma ptau181 is significant in PET A β -positive cases, even without cognitive impairment [90]. Higher ptau181 is associated with increased standardized uptake value ratio of tau PET in Braak I–IV regions of interest [89]. Furthermore, our meta-analysis results reflected the high diagnostic accuracy of plasma tau (AUC 0.77, 95% CI 0.74–0.81) and ptau181 (AUC 0.93, 95% CI 0.91–0.95), similar to the diagnostic accuracy of tau PET (AUC 0.98, 95% CI 0.94–1.00) and CSF ptau181 (AUC 0.97, 95% CI 0.92–1.00) [90]. In contrast, there is only a weak association between CSF and plasma tau, as confirmed by two independent studies



[46, 65], suggesting that they may go through different metabolism. There are no significant differences in plasma or serum A β between AD and controls, and plasma A β may reflect peripheral A β generation instead of brain pathology [95].

In addition, the current meta-analysis supports plasma ptau181 as a better predictive biomarker than plasma tau for AD. The difference in plasma ptau181 between AD and controls was greater than plasma tau in our analysis (WMD: 11.68 pg/ml vs 0.83 pg/ml), and the pooled diagnostic accuracy of ptau181 was also higher (AUC: 0.93 vs 0.77). These are consistent with a previous report that the plasma ptau181 is more strongly associated with both A β and tau PET than plasma tau [60], and can differentiate AD from non-AD pathologies with high accuracy (AUC 97.4, 95% CI 94.1–100%) eight years before death [87], collectively highlighting the potential of ptau181 as a biomarker for AD pathology.

We also found a higher level of plasma ptau181 compared to total tau in the meta-analysis. Phosphorylation is a post-translational modification of the protein, and theoretically, ptau181 should be a portion of total tau in any given tissue [96]. However, according to our analysis of publications based on the Simoa method, the mean level of ptau181 was higher than total tau (11.18 pg/ml vs 3.07 pg/ml). There could be differences in calibration standard, and at this stage, it is not feasible to compare the calculated values of tau and ptau181.

Limitations

There were some limitations in this systematic review and meta-analysis. The ultrasensitive measurement of plasma tau has yet to be extensively tested, leading to high heterogeneity in the results. Meta-regression analyses suggested that the heterogeneity was partly explained by the sample size of studies included. In addition, most of the studies included in the current analysis used clinical diagnostic guidelines rather than gold-standard autopsy-confirmed AD, which can induce heterogeneity. One study [57] on neuropathologically confirmed autopsy cases has revealed a greater change in plasma tau (WMD 5.81), compared to the results from the clinically diagnosed cases (WMD 0.83), highlighting the potential error induced by diagnosis. Even the diagnosis is correct, AD itself can be heterogeneous. For example, familial cohorts [86] may lead to different results from sporadic cohorts.

On the other hand, due to the lack of information on ApoE4 status, we could not measure the impact of ApoE4 on plasma tau and ptau, leading to potential heterogeneity. We have considered age as a factor for heterogeneity, but due to the limited data, we can only analyze the effect of age in healthy populations, where we found no effect of age on plasma tau. Future analysis should be performed when there are more studies on early-onset AD.

We excluded a few publications for analysis. Two using the Simoa platform were excluded as the methods used in those studies had not been validated in multiple cohorts or studies [97], or were specifically validated for CSF (not plasma) [61]. Although results of the two studies are consistent with our findings, it is challenging to combine them with those using the commercialized plasma tau detection kit developed by Quanterix. A few other studies were focused on the serum tau or ptau [98, 99], and were also excluded from our analysis.

We have to mention that some studies on ptau181 were recently published during the final revision of the manuscript, and were not included. These latest studies have investigated the dynamic changes of plasma ptau181 across the AD spectrum [100] and the relationship between polygenic risk scores for AD and plasma ptau181 [101]. They have also compared performance of ptau181 with other biomarkers in AD and MCI prediction [102] or amyloid PET status prediction [103]. In addition, ptau217 [104] and ptau231 (Ashton et al. in press) have been reported recently to differentiate AD from other neurodegenerative disorders and be associated with tau pathology in the brain. The plasma ptau217 may out-perform ptau181 with a higher AUC and stronger correlations with the tau PET [9, 104]. However, here we did not include plasma ptau217 and ptau231 studies in our meta-analysis, due to the limited number of publications.

Conclusion

In summary, the results presented provide preliminary evidence of plasma tau and its phosphorylated form ptau181 as potential biomarkers for neurological diseases, especially for AD diagnosis, which may facilitate drug discovery of these diseases by selecting correct patients for clinical trials.

Abbreviations

AD: Alzheimer's disease; ptau181: Phosphorylated tau 181; AUC: Area under curve; ROC: Receiver operating characteristic; NIA-AA: National Institute on Aging—Alzheimer's Association; A β : Amyloid-beta; PET: Positron emission tomography; NFT: Neurofibrillary tangle; CSF: Cerebrospinal fluid; Simoa: Single-molecule Array; iMR: ImmunoMagnetic Reduction; α -EIMA: E-muB arrayed fiber optics conjugated with rolling circle amplification; MSD: Meso Scale Discovery; NOS: Newcastle-Ottawa Scale; QUADAS-2: Quality Assessment of Diagnostic Accuracy Studies-2 tool; WMD: Weighted mean difference; DOR: Diagnostic odds ratio; HsROC: Hierarchical summary receiver operating characteristic.

Additional Files

Additional file 1 Table S1. The Newcastle-Ottawa Scale (NOS) for assessing the quality of studies in meta-analyses. **Table S2.** Meta-regression results. **Table S3.** Summary of estimate values of diagnostic accuracy.

Additional file 2 Fig. S1. Quality assessment results of included articles. **Fig. S2.** The levels of plasma tau and ptau181 in healthy people using

Simoa in different subgroups. **Fig. S3.** The level of plasma tau in healthy people using IMR in different subgroups. **Fig. S4.** The levels of plasma tau and ptau181 in healthy people using EIMAF/a-EIMAF and MSD. **Fig. S5.** Comparison of plasma tau and ptau181 between AD and healthy controls using Simoa, IMR, and MSD. **Fig. S6.** Funnel plot of the random-effect analysis. **Fig. S7.** The diagnostic accuracy of plasma ptau181 using MSD.

Acknowledgments

Not applicable.

Authors' contributions

PL contributed to the conception, project design, and data interpretation. XLD and STZ performed literature research and drafted the manuscript. LW and CJ helped to collect the data and performed statistical analyses. TL contributed to data interpretation. All authors edited and approved the manuscript.

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

- Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement*. 2018;14(4):535–62.
- Guo T, Zhang D, Zeng Y, Huang TY, Xu H, Zhao Y. Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's disease. *Mol Neurodegener*. 2020;15(1):40.
- Lei P, Ayton S, Finkelstein DL, Spoorri L, Cicciostoto GD, Wright DK, et al. Tau deficiency induces parkinsonism with dementia by impairing APP-mediated iron export. *Nat Med*. 2012;18(7):291–5.
- Lei P, Ayton S, Moon S, Zhang Q, Volitakis I, Finkelstein DL, et al. Motor and cognitive deficits in aged tau knockout mice in two background strains. *Mol Neurodegener*. 2014;9(1):29.
- Lei P, Ayton S, Appukuttan AT, Moon S, Duce JA, Volitakis I, et al. Lithium suppression of tau induces brain iron accumulation and neurodegeneration. *Mol Psychiatry*. 2017;22(3):396–406.
- Tuo QZ, Lei P, Jackman KA, Li XL, Xiong H, Li XL, et al. Tau-mediated iron export prevents ferroptotic damage after ischemic stroke. *Mol Psychiatry*. 2017;22(11):1520–30.
- Shen LL, Manukat-Tan NB, Gao SH, Li WW, Zeng F, Zhu C, et al. The ProNGF/p75NTR pathway induces tau pathology and is a therapeutic target for FTLD-tau. *Mol Psychiatry*. 2018;23(8):1813–24.
- Dage JL, Wennberg AMV, Airey DC, Hagen CE, Knopman DS, Machulda MM, et al. Levels of tau protein in plasma are associated with neurodegeneration and cognitive function in a population-based elderly cohort. *Alzheimers Dement*. 2016;12(12):1226–34.
- Janelidze S, Stomrud E, Smith R, Palmqvist S, Mattsson N, Airey DC, et al. Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. *Nat Commun*. 2020;11(1):1683.
- Barthelemy NR, Li Y, Joseph-Mathurin N, Gordon BA, Hassenstab J, Benzinger TLS, et al. A soluble phosphorylated tau signature links tau, amyloid and the evolution of stages of dominantly inherited Alzheimer's disease. *Nat Med*. 2020;26(3):398–407.
- Karlari TK, Emeršić A, Villion A, Lantero-Rodriguez J, Ashton NJ, Kramberger MG, et al. Head-to-head comparison of clinical performance of CSF phospho-tau T181 and T217 biomarkers for Alzheimer's disease diagnosis. *Alzheimers Dement*. 2020. <https://doi.org/10.1002/alz.12210>.
- Meng J, Lei P. Plasma ptau181 as a biomarker for Alzheimer's disease. *MedComm*. 2020;1(1):74–6.
- Rissin DM, Kan CW, Campbell TG, Hovess SC, Fournier DH, Song L, et al. Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. *Nat Biotechnol*. 2010;28(6):595–9.
- Hong CY, Wu CC, Chiu YC, Yang SY, Horng HE, Yang HC. Magnetic susceptibility reduction method for magnetically labeled immunoassay. *Appl Phys Lett*. 2006;88(2):162.
- Rubenstein R, Chang B, Davies P, Wagner AK, Robertson CS, Wang KK. A novel, ultrasensitive assay for tau: potential for assessing traumatic brain injury in tissues and biofluids. *J Neurotrauma*. 2015;32(5):342–52.
- Zu YB, Bard AJ. Electrogenic chemiluminescence. 66. The role of direct coreactant oxidation in the ruthenium (II) 2,2'-bipyridyl/triethylamine system and the effect of halide ions on the emission intensity. *Anal Chem*. 2000;72(14):3223–32.
- Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. 2009;6(7):e1000097.
- McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;7(3):263–9.
- McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984;34(7):939–44.
- Wells GA, Shea B, O'Connell D, Peterson J, Welch V, et al. The Newcastle-Ottawa scale (NOS) for assessing the quality of non-randomised studies in meta-analyses. In: 3rd Symposium on Systematic Reviews: Beyond the Basics: July 3–5 2000. Oxford: 2000.
- van Houwelingen HC, Arends LR, Stijnen T. Advanced methods in meta-analysis: multivariate approach and meta-regression. *Stat Med*. 2002;21(4):589–624.
- Harbord RM, Whiting P. Metandi: meta-analysis of diagnostic accuracy using hierarchical logistic regression. *Stata J*. 2009;9(2):211–29.
- Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med*. 2002;21(11):1539–58.
- Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ*. 2003;327(7414):557–60.
- Sterne JA. Meta-analysis in Stata: an updated collection from the Stata journal. *StataCorp LP*; 2009.

26. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med*. 2011;155(8):529–36.
27. Shahim P, Tegner Y, Wilson DH, Randall J, Skilbeck T, Pazooki D, et al. Blood biomarkers for brain injury in concussed professional ice hockey players. *JAMA Neurol*. 2014;71(6):684–92.
28. Bogoslovsky T, Wilson D, Chen Y, Fanlon D, Gill J, Jeromin A, et al. Increases of plasma levels of glial fibrillary acidic protein, tau, and amyloid beta up to 90 days after traumatic brain injury. *J Neurotrauma*. 2017;34(1):66–73.
29. Olivera A, Lejbman N, Jeromin A, French LM, Kim HS, Cashion A, et al. Peripheral total tau in military personnel who sustain traumatic brain injuries during deployment. *JAMA Neurol*. 2015;72(10):1109–16.
30. Oliver JM, Jones MT, Anzalone AJ, Kirk KM, Gable DA, Repshas JT, et al. A season of American football is not associated with changes in plasma tau. *J Neurotrauma*. 2017;34(23):3295–300.
31. Aloisco ML, Tripodis Y, Jamagin J, Baugh CM, Martin B, Chai SS, et al. Repetitive head impact exposure and later-life plasma total tau in former National Football League players. *Alzheimers Dement (Amst)*. 2016;7:33–40.
32. Mielke MM, Hagen CE, Wennberg AMV, Airey DC, Savica R, Knopman DS, et al. Association of plasma total tau level with cognitive decline and risk of mild cognitive impairment or dementia in the Mayo Clinic study on aging. *JAMA Neurol*. 2017;74(9):1073–80.
33. Muller S, Preische O, Gopfert JC, Yanez VAC, Joos TO, Boecker H, et al. Tau plasma levels in subjective cognitive decline: results from the DELCODE study. *Sci Rep*. 2017;7(1):9529.
34. Kasai T, Tatebe H, Kondo M, Ishii R, Ohnishi T, Yeung WTE, et al. Increased levels of plasma total tau in adult Down syndrome. *PLoS One*. 2017;12(1):e0188802.
35. Foiani MS, Woolcott IO, Heller C, Bocchetta M, Heslegrave A, Dick KM, et al. Plasma tau is increased in frontotemporal dementia. *J Neurol Neurosurg Psychiatry*. 2018;89(8):804–7.
36. Bergman L, Zetterberg H, Kaihola H, Hagberg H, Blennow K, Akerud H. Blood-based cerebral biomarkers in preeclampsia: plasma concentrations of NfL , tau, S100B and NSF during pregnancy in women who later develop preeclampsia – a nested case control study. *PLoS One*. 2018;13(5):e0196025.
37. Lippa SM, Yeh PH, Gill J, French LM, Brickell TA, Lange RT. Plasma tau and amyloid are not reliably related to injury characteristics, neuropsychological performance, or white matter integrity in service members with a history of traumatic brain injury. *J Neurotrauma*. 2019;36(14):2190–9.
38. Verberk IMW, Slor RE, Verfaillie SCJ, Heijst H, Fris ND, van Berckel BNM, et al. Plasma amyloid as prescanner for the earliest Alzheimer pathological changes. *Ann Neurol*. 2018;84(5):648–58.
39. Shahim P, Tegner Y, Marklund N, Blennow K, Zetterberg H. Neurofilament light and tau as blood biomarkers for sports-related concussion. *Neurology*. 2018;90(20):e1780–8.
40. Wallace C, Zetterberg H, Blennow K, van Donkelaar P. No change in plasma tau and serum neurofilament light concentrations in adolescent athletes following sport-related concussion. *PLoS One*. 2018;13(1):e0206466.
41. Motamed V, Kanefsky R, Marangas P, Mithani S, Jeromin A, Brock MS, et al. Elevated tau and interleukin-6 concentrations in adults with obstructive sleep apnea. *Sleep Med*. 2018;43:71–6.
42. Fortea J, Carmona-Iragui M, Benjaim B, Fernandez S, Videla L, Barceta I, et al. Plasma and CSF biomarkers for the diagnosis of Alzheimer's disease in adults with Down syndrome: a cross sectional study. *Lancet Neurol*. 2018;17(10):880–9.
43. Zetterberg H, Thomas-Brock G, Garcia-Moreno H, Foiani M, Heslegrave A, Zetterberg H, et al. Plasma markers of neurodegeneration are raised in Friedrich's ataxia. *Front Cell Neurosci*. 2018;12:366.
44. Shi M, Kovac A, Korfi A, Cook TJ, Ghingina C, Bullock KM, et al. CNS tau efflux via exosomes is likely increased in Parkinson's disease but not in Alzheimer's disease. *Alzheimers Dement*. 2016;12(11):125–31.
45. Karikari TK, Benedict AL, Ashton NJ, Lantero-Rodriguez J, Snellman A, Suarez-Calvet M, et al. Diagnostic performance and prediction of clinical progression of plasma phospho-tau181 in the Alzheimer's disease neuroimaging initiative. *Mol Psychiatry*. 2021;26(2):429–42.
46. Pase MP, Beiser AS, Himel JJ, Satizabal CL, Aparicio HJ, DeCarli C, et al. Assessment of plasma total tau level as a predictive biomarker for dementia and related endophenotypes. *JAMA Neurol*. 2019;76(5):598.
47. Korley FK, Yue JK, Wilson DH, Hrusovsky K, Diaz-Arriaza R, Ferguson AR, et al. Performance evaluation of a multiplex assay for simultaneous detection of four clinically relevant traumatic brain injury biomarkers. *J Neurotrauma*. 2018;35(1):182–7.
48. Krinkos M, Clouston SAP, Diminich ED, Deri Y, Yang X, Carr M, et al. Pathway analysis for plasma β -amyloid, tau and neurofilament light (ATN) in world trade center responders at midlife. *Neurol Ther*. 2020;9(1):159–71.
49. de Wolf F, Ghanbari M, Licher S, McKee-McKee K, Gras L, Weverling GJ, et al. Plasma tau, neurofilament light chain and amyloid- β levels and risk of dementia: a population-based cohort study. *Brain*. 2020;143(4):1220–32.
50. Verberk IMW, Hendriksen HMA, van Harten AC, Wesselman LMP, Verfaillie SCJ, van den Bosch KA, et al. Plasma amyloid is associated with the rate of cognitive decline in cognitively normal elderly: the SCIENCE project. *Neurobiol Aging*. 2020;89:99–107.
51. Pattinson CL, Gill JM, Lippa SM, Brickell TA, French LM, Lange RT. Concurrent mild traumatic brain injury and posttraumatic stress disorder is associated with elevated tau concentrations in peripheral blood plasma. *J Trauma Stress*. 2019;32(4):546–54.
52. Romero JR, Demisse S, Beiser AS, Himel JJ, DeCarli C, Levy D, et al. Relation of plasma β -amyloid, clusterin, and tau with cerebral microbleeds: Framingham heart study. *Ann Clin Transl Neurol*. 2020;7(7):1083–91.
53. Petersen ME, Rafii MS, Zhang F, Hall J, Julovich D, Ances BM, et al. Plasma total-tau and neurofilament light chain as diagnostic biomarkers of Alzheimer's disease dementia and mild cognitive impairment in adults with Down syndrome. *J Alzheimers Dis*. 2021;79(2):671–81.
54. Cantero JL, Alenza M, Ramos-Cejudo J, Fossati S, Wisniewski T, Osorio RS. Plasma tau predicts cerebral vulnerability in aging. *Aging*. 2020;12(21):21004–22.
55. Zetterberg H, Wilson D, Andreasson U, Minthon L, Blennow K, Randall J, et al. Plasma tau levels in Alzheimer's disease. *Alzheimers Res Ther*. 2013; 5(2):9.
56. Mattsson N, Zetterberg H, Janelidze S, Insel PS, Andreasson U, Stenlund FL, et al. Plasma tau in Alzheimer disease. *Neurology*. 2016;87(17):1827–35.
57. Kovacs GG, Andreasson U, Liman V, Regelsberger G, Lutz M, Danics K, et al. Plasma and cerebrospinal fluid tau and neurofilament concentrations in rapidly progressive neurological syndromes: a neuropathology-based cohort. *Eur J Neurol*. 2017;24(11):1326–e77.
58. Deits KD, Bischoff SL, Kim S, Nho K, West JD, Blennow K, et al. Plasma tau association with brain atrophy in mild cognitive impairment and Alzheimer's disease. *J Alzheimers Dis*. 2017;58(4):1245–54.
59. Chen J, Yu JT, Wojta K, Wang HF, Zetterberg H, Blennow K, et al. Genome-wide association study identifies MAPT locus influencing human plasma tau levels. *Neurology*. 2017;88(7):669–76.
60. Mielke MM, Hagen CE, Xu J, Chai X, Vermeir P, Lowe VJ, et al. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. *Alzheimers Dement*. 2018;14(8):989–97.
61. Park JC, Han SH, Yi D, Byun MS, Lee JH, Jang S, et al. Plasma tau/amyloid-beta1-42 ratio predicts brain tau deposition and neurodegeneration in Alzheimer's disease. *Brain*. 2019;142(3):771–86.
62. Li WW, Shen YY, Tian DY, Bu XL, Zeng F, Liu YH, et al. Brain amyloid-beta deposition and blood biomarkers in patients with clinically diagnosed Alzheimer's disease. *J Alzheimers Dis*. 2019;69(1):169–78.
63. Startin CM, Ashton NJ, Hamburg S, Hithersay R, Wiseman FK, Mok KY, et al. Plasma biomarkers for amyloid, tau, and cytokines in Down syndrome and sporadic Alzheimer's disease. *Alzheimers Res Ther*. 2019;11(1):26.
64. Sugaman MA, Zetterberg H, Blennow K, Tripodis Y, McKee AC, Stein TD, et al. A longitudinal examination of plasma neurofilament light and total tau for the clinical detection and monitoring of Alzheimer's disease. *Neurobiol Aging*. 2020;94:60–70.
65. Fossati S, Ramos-Cejudo J, Debut L, Pirraglia E, Soné JY, Li Y, et al. Plasma tau complements CSF tau and P-tau in the diagnosis of Alzheimer's disease. *Alzheimers Dement (Amst)*. 2019;11:483–92.
66. Deniz K, Ho CCG, Malphrus KG, Reddy JS, Nguyen T, Camrath TP, et al. Plasma biomarkers of Alzheimer's disease in African Americans. *J Alzheimers Dis*. 2021;79(1):323–34.
67. Lin CH, Yang SY, Hsiao HE, Yang CC, Chieh JJ, Chen HH, et al. Plasma biomarkers differentiate Parkinson's disease from atypical parkinsonism syndromes. *Front Aging Neurosci*. 2018;10:123.
68. Chi NF, Chao SP, Huang LK, Chan L, Chen YR, Chiou HY, et al. Plasma amyloid beta and tau levels are predictors of post-stroke cognitive impairment: a longitudinal study. *Front Neurol*. 2019;10:715.
69. Chen TB, Lee YJ, Lin SY, Chen JP, Hu CJ, Wang PN, et al. Plasma A β 42 and total tau predict cognitive decline in amnesic mild cognitive impairment. *Sci Rep*. 2019;9(1):13984.

70. Chen NC, Chen HL, Li SH, Chang YH, Chen MH, Tsai NW, et al. Plasma levels of α -synuclein, A β -40 and T-tau as biomarkers to predict cognitive impairment in Parkinson's disease. *Front Aging Neurosci.* 2020;12:112.
71. Fang WQ, Hwu WL, Chien YH, Yang SY, Chieh JJ, Chang LM, et al. Composite scores of plasma tau and β -amyloids correlate with dementia in Down syndrome. *ACS Chem Neurosci.* 2020;11(2):391–6.
72. Chiu MJ, Fan LY, Chen TF, Chen YF, Chieh JJ, Hsing HE. Plasma tau levels in cognitively normal middle-aged and older adults. *Front Aging Neurosci.* 2017;9:51.
73. Chiu MJ, Chen YF, Chen TF, Yang SY, Yang EP, Tseng TW, et al. Plasma tau as a window to the brain: negative associations with brain volume and memory function in mild cognitive impairment and early Alzheimer's disease. *Hum Brain Mapp.* 2014;35(7):3132–42.
74. Tzen KY, Yang SY, Chen TF, Cheng TW, Hsing HE, Wen HP, et al. Plasma A β but not tau is related to brain β B retention in early Alzheimer's disease. *ACS Chem Neurosci.* 2014;5(9):830–6.
75. Yang SY, Chiu MJ, Chen TF, Lin CH, Jeng JS, Tang SC, et al. Analytical performance of reagent for assaying tau protein in human plasma and feasibility study screening neurodegenerative diseases. *Sci Rep.* 2017;7(1):9304.
76. Lee NC, Yang SY, Chieh JJ, Huang PT, Chang LM, Chiu YN, et al. Blood beta-amyloid and tau in Down syndrome: a comparison with Alzheimer's disease. *Front Aging Neurosci.* 2016;8:316.
77. Lue LF, Sabbagh MN, Chiu MJ, Jing N, Snyder NL, Schmitz C, et al. Plasma levels of A β 42 and tau identified probable Alzheimer's dementia: findings in two cohorts. *Front Aging Neurosci.* 2017;9:226.
78. Yang CC, Chiu MJ, Chen TF, Chang HL, Liu BH, Yang SY. Assay of plasma phosphorylated tau protein (threonine 181) and total tau protein in early-stage Alzheimer's disease. *J Alzheimers Dis.* 2018;61(4):323–32.
79. Chiu MJ, Lue LF, Sabbagh MN, Chen TF, Chen HH, Yang SY. Long-term storage effects on stability of A β 1–40, A β 1–42, and total tau proteins in human plasma samples measured with immunomagnetic reduction assays. *Dement Geriatr Cogn Dis Extra.* 2019;9(1):77–86.
80. Jiao F, Yi F, Wang Y, Zhang S, Guo Y, Du W, et al. The validation of multifactor model of plasma A β (42) and total tau in combination with MoCA for diagnosing probable Alzheimer disease. *Front Aging Neurosci.* 2020;12:212.
81. Liu HC, Chiu MJ, Lin CH, Yang SY. Stability of plasma amyloid β 1–40, amyloid β 1–42, and total tau protein over repeated freeze/thaw cycles. *Dement Geriatr Cogn Dis Extra.* 2020;10(1):46–55.
82. Rubenstein R, Chang B, Yue JK, Chiu A, Winkler EA, Puccio AM, et al. Comparing plasma phospho-tau, total tau, and phospho-tau-total tau ratio as acute and chronic traumatic brain injury biomarkers. *JAMA Neurol.* 2017;74(9):1063–72.
83. Gardner RC, Rubenstein R, Wang KKW, Korley FK, Yue JK, Yuh EL, et al. Age-related differences in diagnostic accuracy of plasma glial fibrillary acidic protein and tau for identifying acute intracranial trauma on computed tomography: a TRACK-TBI study. *J Neurotrauma.* 2018;35(20):2341–50.
84. Suárez-Calvet M, Karikari TK, Ashton NJ, Lantero Rodríguez J, Milà-Alomà M, Gispert JC, et al. Novel tau biomarkers phosphorylated at T181, T217 or T231 rise in the initial stages of the preclinical Alzheimer's continuum when only subtle changes in A β pathology are detected. *EBioMedicine.* 2020;13(1):2e12921.
85. Moscoso A, Grothe MJ, Ashton NJ, Karikari TK, Lantero Rodríguez J, Snellman A, et al. Longitudinal associations of blood phosphorylated tau181 and neurofilament light chain with neurodegeneration in Alzheimer disease. *JAMA Neurol.* 2021. <https://doi.org/10.1001/jamaneurol.2020.4986>.
86. O'Connor A, Karikari TK, Poole T, Ashton NJ, Lantero Rodríguez J, Khatun A, et al. Plasma phospho-tau181 in presymptomatic and symptomatic familial Alzheimer's disease: a longitudinal cohort study. *Mol Psychiatry.* 2020. <https://doi.org/10.1038/s41380-020-0239-x>.
87. Lantero Rodríguez J, Karikari TK, Suárez-Calvet M, Troakes C, King A, Emerick A, et al. Plasma p-tau181 accurately predicts Alzheimer's disease pathology at least 8 years prior to post-mortem and improves the clinical characterisation of cognitive decline. *Acta Neuropathol.* 2020;140(3):267–78.
88. Karikari TK, Pascoal TA, Ashton NJ, Janelidze S, Benedet AL, Rodríguez JL, et al. Blood phosphorylated tau (181) as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol.* 2020;19(5):422–33.
89. Thijssen EH, La Joie R, Wolf A, Ström A, Wang P, Iaccarino L, et al. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. *Nat Med.* 2020;26(3):387–97.
90. Janelidze S, Mattsson N, Palmqvist S, Smith R, Beach TG, Serrano GE, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat Med.* 2020;26(3):379–86.
91. Kitaguchi N, Tatebe H, Sakai K, Kawaguchi K, Matsunaga S, Kitajima T, et al. Influx of tau and amyloid-beta proteins into the blood during hemodialysis as a therapeutic extracorporeal blood amyloid-beta removal system for Alzheimer's disease. *J Alzheimers Dis.* 2019;69(3):687–707.
92. Oliver JM, Anzalón AJ, Stone JD, Turner SM, Bluet D, Garrison JC, et al. Fluctuations in blood biomarkers of head trauma in NCAA football athletes over the course of a season. *J Neurosurg.* 2019;130(5):1655–62.
93. Shi Y, Lu X, Zhang L, Shu H, Gu L, Wang Z, et al. Potential value of plasma amyloid-beta, total tau, and neurofilament light for identification of early Alzheimer's disease. *ACS Chem Neurosci.* 2019;10(8):3479–85.
94. Arvanitakis Z, Shah RC, Bennett DA. Diagnosis and management of dementia: review. *JAMA.* 2019;322(16):1589–99.
95. Olsson B, Lautner R, Andreasson U, Ohrlert A, Pernelius E, Björke M, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol.* 2016;15(7):673–84.
96. Wang HY, Bakshi K, Frankfurt M, Stucky A, Gøberdhan M, Shah SM, et al. Reducing amyloid-related Alzheimer's disease pathogenesis by a small molecule targeting flamin A. *J Neurosci.* 2012;32(29):9773–84.
97. Tatebe H, Kasai T, Ohnishi T, Kishi Y, Kakeya T, Waragai M, et al. Quantification of plasma phosphorylated tau to use as a biomarker for brain Alzheimer pathology: pilot case-control studies including patients with Alzheimer's disease and Down syndrome. *Mol Neurodegener.* 2017;12(1):63.
98. Benussi A, Karikari TK, Ashton N, Gazzina S, Premi F, Benussi L, et al. Diagnostic and prognostic value of serum NfL and p-tau (181) in frontotemporal lobar degeneration. *J Neurol Neurosurg Psychiatry.* 2020;91(9):960–7.
99. Shekhar S, Kumar R, Bai N, Kumar V, Singh K, Upadhyay AD, et al. Estimation of tau and phosphorylated Tau181 in serum of Alzheimer's disease and mild cognitive impairment patients. *PLoS One.* 2016;11(7):e0159099.
100. Moscoso A, Grothe MJ, Ashton NJ, Karikari TK, Rodríguez JL, Snellman A, et al. Time course of phosphorylated tau181 in blood across the Alzheimer's disease spectrum. *Brain.* 2021;144(7):325–39.
101. Zettergren A, Lord J, Ashton NJ, Benedet AL, Karikari TK, Lantero Rodríguez J, et al. Association between polygenic risk score of Alzheimer's disease and plasma phosphorylated tau in individuals from the Alzheimer's disease neuroimaging initiative. *Alzheimers Res Ther.* 2021;13(1):7.
102. Simón J, Leuzay A, Karikari TK, Hye A, Benedet AL, Lantero-Rodriguez J, et al. The diagnostic and prognostic capabilities of plasma biomarkers in Alzheimer's disease. *Alzheimers Dement.* 2021. <https://doi.org/10.1002/alz.12283>.
103. Keshavan A, Pannee J, Karikari TK, Rodríguez JL, Ashton NJ, Nicholas JM, et al. Population-based blood screening for preclinical Alzheimer's disease in a British birth cohort at age 70. *Brain.* 2021;awan403. <https://doi.org/10.1093/brain/awz403>.
104. Palmqvist S, Janelidze S, Quiroz YT, Zetterberg H, Lopera F, Stomrud E, et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs. other neurodegenerative disorders. *JAMA.* 2020;324(8):772–81.

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APPENDIX C: Ding et al., Scientific Commentaries. Brain (2020)

SCIENTIFIC COMMENTARIES

Plasma p-tau217: from ‘new kid’ to most promising candidate for Alzheimer’s disease blood test

This scientific commentary refers to ‘Longitudinal plasma p-tau217 is increased in early stages of Alzheimer’s disease’, by Mattsson-Carlgren *et al.* (doi:10.1093/brain/awaa286).

Alzheimer’s disease is characterized by a long presymptomatic phase during which neuropathology gradually accumulates. Detecting this pathology at the earliest possible stage offers the best chance of effective treatment. Clinical trials targeting the key pathological hallmarks of Alzheimer’s disease, such as cerebral amyloid- β aggregation and tangle formation, therefore focus on enrolling non-demented individuals with early-stage biomarker-defined disease. A reliable blood test for Alzheimer’s disease would have major implications for the selection of clinical trial participants by allowing non-demented individuals to be pre-screened in a minimally invasive and cost-effective manner, reducing the numbers who must undergo further testing with more invasive and expensive measures such as CSF analyses or PET scans.

Studies analysing plasma amyloid have shown that pre-screening with a blood test can significantly reduce further testing with more invasive measures (Verberk *et al.*, 2018), while more recent evidence suggests that measures of plasma phosphorylated tau (p-tau) may have greater diagnostic power than plasma amyloid measures

(Janelidze *et al.*, 2020; Thijssen *et al.*, 2020). Cross-sectional studies covering the complete clinical Alzheimer’s disease continuum have shown that plasma isoforms p-tau181 and p-tau217 can differentiate amyloid-PET or tau-PET positive cases from amyloid-PET or tau-PET negative cases (Janelidze *et al.*, 2020; Karikari *et al.*, 2020; Palmqvist *et al.*, 2020; Thijssen *et al.*, 2020). These cross-sectional studies have also shown that plasma p-tau measures can distinguish patients with Alzheimer’s disease dementia from those with frontotemporal lobar degeneration (Janelidze *et al.*, 2020; Karikari *et al.*, 2020; Palmqvist *et al.*, 2020; Thijssen *et al.*, 2020). In this issue of *Brain*, a timely longitudinal study by Mattsson-Carlgren and co-workers extends this work to the preclinical stages of Alzheimer’s disease, and shows the value of using p-tau217 for participant selection in clinical trials as well as for disease monitoring (Mattsson-Carlgren *et al.*, 2020).

Mattsson-Carlgren *et al.* included 250 non-demented individuals from the Swedish BioFINDER study, and measured p-tau217 levels at baseline and during follow-up using the ‘Meso Scale Discovery’ (MSD) Eli Lilly immunoassay. The results showed that p-tau217 was increased in individuals in the preclinical and early clinical stages of Alzheimer’s disease when compared to cognitively healthy controls. In addition, higher p-tau217

levels were associated with a greater risk of converting to Alzheimer’s disease dementia and with steeper rates of cognitive decline and thinning of the temporal cortex and hippocampus. As well as showing that p-tau217 could be used to identify participants for inclusion in clinical trials, the results also suggest that p-tau217 could be used to monitor treatment responses over time. The authors showed that p-tau217 levels increased more steeply in non-demented individuals with Alzheimer’s disease pathology (amyloid positivity) than in those with no evidence of such pathology (Fig. 1). p-Tau217 levels also rose more steeply in non-demented individuals who developed Alzheimer’s disease dementia compared to those who remained non-demented during follow-up (Fig. 1). The logical next step would therefore be to analyse p-tau217 in blood samples obtained during trials that show reduction or even complete clearance of amyloid in the brain (Seigney *et al.*, 2016) to determine whether p-tau217 could help monitor treatment response.

Results to date suggest that p-tau217 could also play a major role in a clinical diagnostic setting. For a biomarker to be used in the clinic, three phases of development must be completed (Fig. 2). The first phase, which focuses on assay development and validation plus initial clinical validation, has almost been completed for p-tau217. The Eli Lilly assay has shown robust

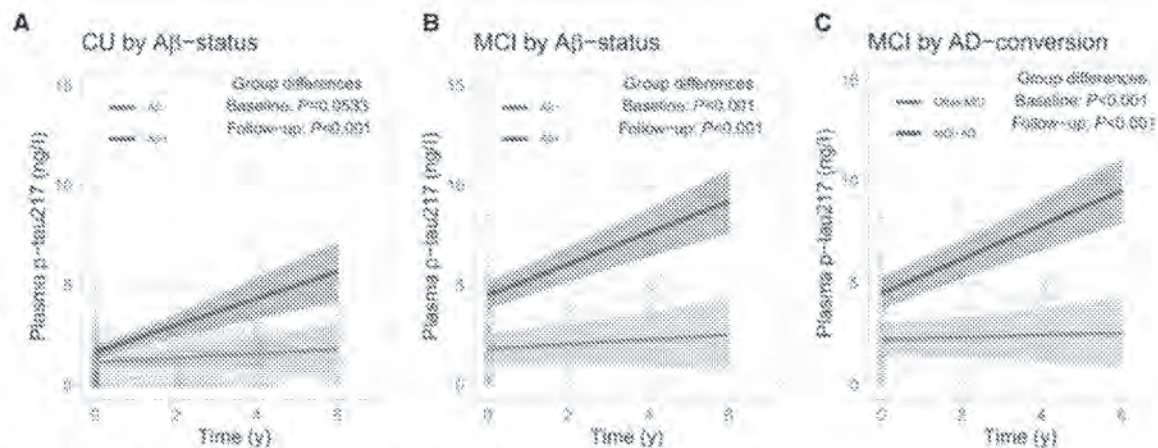


Figure 1 Longitudinal plasma p-tau217 data from Mattsson-Carligen *et al.* (2020). Subject-specific biomarker data are shown together with main effects from linear mixed effects models, adjusted for age and sex, for p-tau217 in (A) amyloid- β -negative (A β -) cognitively unimpaired (CU) versus amyloid- β -positive (A β +) cognitively unimpaired; (B) amyloid- β -negative mild cognitive impairment (MCI) versus amyloid- β -positive MCI; and (C) MCI to Alzheimer's disease (AD) dementia converters versus the remaining MCI population (i.e. stable MCI or 'MCI to other dementia' converters).

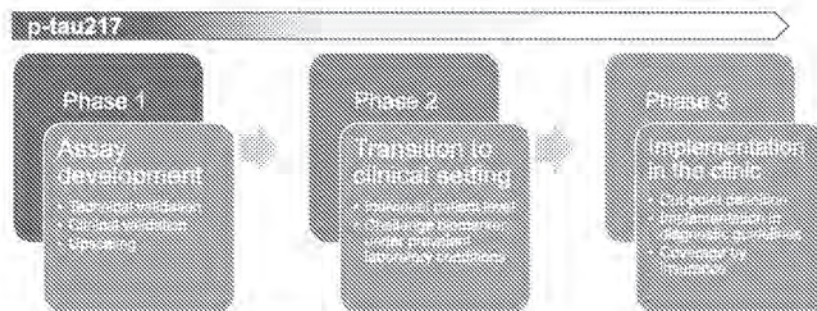


Figure 2 Three-phase trajectory of biomarker development.

outcomes (Palmqvist *et al.*, 2020), although the results of assay validation studies have yet to be published. The second phase focuses on the transition from a research setting to a clinical setting, with the aim of ensuring that the results are consistent at the level of individual patients in different settings and to define the context of use. Here, clinical evaluation should focus on establishing large databases containing information on the plasma p-tau217 concentrations of individual patients alongside their clinical and biomarker characteristics. During this phase, work on the assay includes assessing its robustness, developing reference material, and testing biomarker stability under prevalent

conditions. This may include testing the variation introduced by delays in processing samples or by the need to cool samples and transport them to the laboratory. The third phase focuses on the final definition of cut-points based on the data obtained in phase 2, and on evaluation of the assay results in real-life clinical settings in unselected patients. In parallel, insurance coverage of plasma testing for diagnostic purposes needs to be arranged where required, and the biomarker positioned in clinical diagnostic guidelines. Currently, we are close to reaching the end of phase one for p-tau217. It is thus time to transition to phases two and three, aiming for a swift implementation of

the plasma p-tau217 biomarker in both trials and clinical practice, to accelerate drug development and improve clinical care for Alzheimer's disease.

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Competing interests

The authors report no competing interests.

References

- Janelidze S, Mattsson N, Palmqvist S, Smith R, Beach TG, Serrano GE, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat Med* 2020; 26: 379–86.
- Karikari TK, Pascoal TA, Ashton NJ, Janelidze S, Beneder AL, Rodriguez JL, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol* 2020; 19: 422–33.
- Mattsson-Carlgen N, Janelidze S, Palmqvist S, Cullen N, Svenningsson AL, Strandberg O, et al. Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease. *Brain* 2020; 143: 3234–41.
- Palmqvist S, Janelidze S, Quiroz YT, Zetterberg H, Lopera F, Stomrud E, et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. *JAMA* 2020; 324: 772–81.
- Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M, et al. The antibody aducanumab reduces A β plaques in Alzheimer's disease. *Nature* 2016; 537: 50–6.
- Thijssen EH, La Joie R, Wolf A, Siron A, Wang P, Iaccarino L, et al. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. *Nat Med* 2020; 26: 387–97.
- Verberk IMW, Slot RE, Verfaillie SCJ, Heijst H, Prins ND, van Berckel BNM, et al. Plasma amyloid as prescreener for the earliest Alzheimer pathological changes. *Ann Neurol* 2018; 84: 648–58.

Is bad brain worse than no brain? Salvaging the cerebral cortex in epilepsy

This scientific commentary refers to 'Resective surgery prevents progressive cortical thinning in temporal lobe epilepsy', by Galovic *et al.* (doi:10.1093/brain/awaa284).

The human cerebral cortex, whose thickness ranges from 1 to 4 mm depending on the region, has one of the highest neuronal densities of all species. This highly folded sheet of cells is responsible for many of the higher cognitive functions that characterize humans. Cortical thickness, a proxy for cortical structural integrity, is dynamic; it changes with growth, maturation and ageing. However, our increasing ability to assess the cortex *in vivo* has also revealed associations between cortical thickness and factors such as sex, genetic background, cognitive function and disease states. Cortical thinning that progresses at a rate higher than expected for age has been demonstrated in a number of neurological and psychiatric conditions, including schizophrenia, depression, dementia and neurodegenerative disorders, multiple sclerosis and, not least, epilepsy. In this issue of *Brain*, Galovic and co-workers shed new light on this important area of epilepsy research (Galovic *et al.*, 2020).

That loss of brain tissue occurs in chronic epilepsy has been known for many years, as a result of structural brain imaging and the analysis of pathological specimens. What has been less well studied until more recently is the relative contribution of cortical thinning to overall brain tissue loss in epilepsy. Other unanswered questions include the histopathological and pathophysiological underpinnings of cortical thinning, its rate of progression and topography, its association with specific clinical aspects of epilepsy, and importantly, its response to effective epilepsy surgery.

One concept that is closely related to cortical thinning in epilepsy—a process that occurs over time—is that of epilepsy as a progressive disorder. Progressive structural brain changes in epilepsy include neuronal damage and loss, dendritic and synaptic reorganization, axonal loss and thinning, neurodegenerative changes, and gliosis and microgliosis. Progressive functional brain changes in epilepsy include the emergence of the abnormal connectivity and synaptic circuitry that sustains epileptogenesis and that gives rise to abnormal cognitive function. The severity of epilepsy can also increase over time, with the extent of the

increase depending on the type of epilepsy or epilepsy syndrome, and the patient's age and genetic background (Pitkänen and Sutula, 2002).

The notion that seizures themselves contribute to the observed progressive worsening of epilepsy and cognitive function is supported by observations from basic research (Pitkänen and Sutula, 2002). As early as the 19th century, Gowers proposed that 'seizures beget seizures', and in the 1950s, Penfield and Jasper introduced the concept of 'nociferous cortex' in patients with epilepsy (Penfield and Jasper, 1954). This term was applied to cerebral cortex that does not function normally, is the origin of epilepsy, impairs the function of other brain areas, and whose resection results in dramatic improvement of function, behaviour and seizures. Clinical observations, however, have yielded less consistent results with regard to the progressive nature of epilepsy and cognitive decline, and regarding the role of the epileptic process and the nociferous cortex.

Recent advances in quantitative MRI have produced objective imaging biomarkers of disease progression that allow us to measure the rate of age-adjusted cortical thinning in a variety of

APPENDIX 2

Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury

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Abstract—Objective: We investigated if tau, microtubular binding protein, in serum and ventricular CSF (vCSF) in patients with severe traumatic brain injury (TBI) during the initial posttraumatic days correlated to 1-year outcome. **Methods:** Patients with severe TBI ($n = 39$, Glasgow Coma Scale score ≤ 8) were included. We measured serum and vCSF total tau on days 0 to 14, using ELISA. vCSF total tau correlated to 1-year Extended Glasgow Outcome Scale (GOSE), the NIH Stroke Scale (NIHSS) neurologic status, and the Bartel Daily Living Index. Patients ($n = 20$) with normal pressure hydrocephalus (NPH) served as reference. **Results:** Higher levels of tau were found in TBI patients vs patients with NPH. A correlation was found between initial vCSF total tau and GOSE levels ($R = 0.42$, $p < 0.001$) but not between vCSF total tau and NIHSS or Bartel scores at 1 year. A vCSF total tau level of $>2,126$ pg/mL on days 2 to 3 discriminated between dead and alive (sensitivity of 100% and a specificity of 81%). A vCSF total tau level of >702 pg/mL on days 2 to 3 discriminated between bad (GOSE 1 to 4) and good (GOSE 5 to 8) outcome (sensitivity of 83% and a specificity of 69%). Patients with GOSE 1 (dead) had higher vCSF total tau levels on days 2 to 3 ($p < 0.001$) vs both surviving patients (GOSE 2 to 8) and those with NPH. Total tau was not detected in serum throughout the study. **Conclusion:** The increase in ventricular CSF (vCSF) total tau probably reflects axonal damage, known to be a central pathologic mechanism in traumatic brain injury (TBI). These results suggest that vCSF total tau may be an important early biochemical neuromarker for predicting long-term outcome in patients with a severe TBI.

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Different biochemical neuromarkers (BNMs) can reflect the extent of traumatic brain injury (TBI). Among them, S-100 β and neuron-specific enolase (NSE), measured in blood, have most frequently been utilized. Although associations between concentrations of S-100B and outcome in TBI patients are reported, both S-100B and NSE have low sensitivity and specificity.¹ Focus has been oriented toward other BNMs.² We have demonstrated that elevated levels of glial fibrillary acidic protein are associated with bleak 1-year outcome in patients with TBI.³

Different types of cellular damage in TBI, involving different receptors and biochemical pathways, may be identified or at least characterized by BNMs. For prognosis, diffuse axonal injury (DAI) may be the most deleterious response in patients with TBI.⁴ DAI is predominantly noted in discrete regions of the brain following high-speed head rotation or deceleration injuries. DAI can histologically be detected in all unconscious TBI patients.⁵

Tau proteins, with a molecular mass of 48 to 67 kd and mainly found in neurons, are microtubule asso-

ciated and play an important role in the assembly of tubulin monomers into microtubules and in maintaining the cytoskeleton and axonal transport. Aggregation of specific sets of tau proteins in filamentous inclusions is the common feature of intraneuronal tangles.⁶

In this study, we investigated if total tau in serum or ventricular CSF (vCSF) is associated with long-term outcome (1-year) in patients with severe TBI. We hypothesized that higher tau levels could be associated with a more severe TBI and a worse long-term outcome.

Methods. The study was performed in accordance with the provisions of the Helsinki Declaration, and the University Hospital Medical Ethics Committee, Gothenburg, Sweden, approved the study protocol. Informed written consent was obtained from each patient's next of kin. The patients were admitted from October 2000 to December 2002 to the Neurointensive Care Unit (NICU) at Sahlgrenska University Hospital, Gothenburg, Sweden. Patients were included within 48 hours after trauma. The inclusion criteria were severe TBI (i.e., a Glasgow Coma Scale [GCS] score of ≤ 8 at admission), operated with placement of an intracranial ventricular catheter, and a need for artificial ventilation. Serum

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and vCSF was intermittently collected post TBI on days 0 (trauma day), 1 to 4, 6, 8, and once in the period of days 11 to 14.

After clinical and radiologic evaluations, the patients underwent neurosurgical intervention within hours (1 to 4 hours) after admission, to receive an indwelling ventricular catheter for intracranial pressure (ICP) monitoring/therapeutic CSF drainage. When appropriate, space-occupying lesions like hemorrhages and contusions were surgically removed. Patients were then treated in accordance with a standardized protocol, the Lund concept.⁷ Data collected included age, sex, and premorbid health status. Physiologic and laboratory measures were continuously recorded throughout the study period and concomitantly adjusted to be kept within the following limits: hemoglobin >120 g/L, serum sodium >135 to <150 mmol/L, serum potassium 4.0 to 5.0 mmol/L, serum albumin 35 to 50 g/L, core temperature $37 \pm 0.5^\circ\text{C}$, mean arterial blood pressure (MABP) between 70 and 100 mm Hg, ICP <20 mm Hg, cerebral perfusion pressure (= MABP - ICP) >60 mm Hg, Po_2 12 to 18 kPa, Pco_2 around 4.5 kPa, and normalized pH. Blood glucose was kept between 4 and 6 mmol/L according to NICU routine.

CSF and blood samples were centrifuged at $\times 2,000$ g for 10 minutes at 4°C and frozen at -70°C until analyzed. Occasionally vCSF samples could not be obtained because of unstable high ICP.

As a reference group, we examined vCSF from 20 patients with NPH. Details of this patient cohort have previously been presented.⁸

Tau analyses. Total tau (normal tau and hyperphosphorylated tau) was determined using a sandwich ELISA technique (Innotest hTAU-Antigen; Innogenetics N.V., Ghent, Belgium), as previously described in detail.⁹

Neurologic assessment. One year post TBI, an experienced neurologist unaware of neurochemical results assessed neurology on all living patients routinely at the hospital but occasionally at the patient's home. To assess outcome, the Extended Glasgow Outcome Scale (GOSE) was utilized, where 1 = dead and 8 = totally recovered.¹⁰ GOSE could then be dichotomized to bad (GOSE 1 to 4) and good (GOSE 5 to 8) outcome. The severity of the neurologic deficit at 1 year after trauma was assessed with the NIH Stroke Scale (NIHSS), where 0 = no neurologic deficit and 36 = major deficits. Bartel Index was used to rate activities of daily living (ADL), where 100 = fully independent and 0 = patient needs help with all ADL.

Statistical analysis. All statistical procedures were performed using Statview and SAS. Because normal distribution could not be obtained, nonparametric tests (i.e., Kruskal-Wallis one-way analysis of variance and Mann-Whitney) were utilized to investigate group differences. Data are given as medians and interquartile ranges (IQR). For correlations, we used Spearman correlation test. We utilized receiver-operating curve (ROC) to detect the best sensitivity and specificity for 1-year outcome, that is, mortality and morbidity. Area under the curve (AUC) was utilized to further emphasize sensitivity and specificity. Positive likelihood ratio (LR+) was calculated by dividing sensitivity with $(100 - \text{specificity})$. LR+ may be used to find clinical relevant cut-off values. All tests were two tailed, and p values of <0.05 were considered as significant.

Results. Fifty-six patients with TBI were primary included, but unstable ICP only allowed vCSF collection in 39 patients. These patients (women, $n = 9$; men, $n = 30$), with a median age of 49 years (range 16 to 82 years), are those presented.

Three patients had a history of epilepsy, one of which had been operated for meningioma, and three more patients had a previous history of other neurologic diseases like TIA, stroke, or polio. Thus, the remaining 33 patients had no history of neurologic disorders before TBI. Eight patients had measurable content of blood ethanol, and three had narcotics in the blood at the time of accident. The remaining 28 patients had no measurable drug content in the blood at the time of accident; however, 2 of these had a history of alcohol addiction. The initial trauma was traffic accident in 16 patients, fall accidents in 13 patients, and miscellaneous causes like assault in 10 pa-

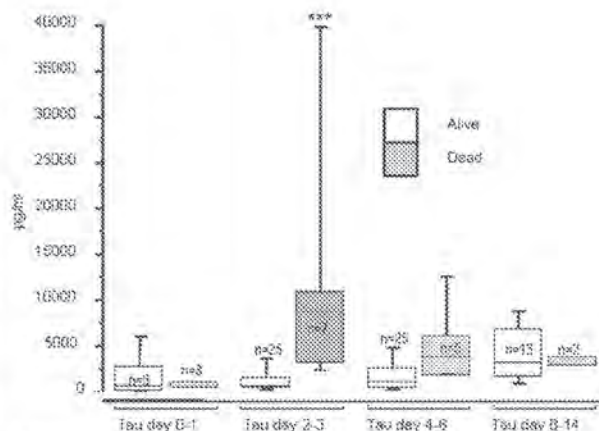


Figure 1. Tau levels in ventricular CSF days 0 to 14 in alive vs dead patients related to 1-year outcome in traumatic brain injury patients. *** $p < 0.001$.

tients. Twenty-six patients had isolated TBI, whereas in eight cases, other fractures than in the skull could also be detected. Finally, in five patients, internal organs were traumatized in combination with TBI.

At hospital arrival, the patients' scores ranged in GCS from 3 to 15, subdividing them as GCS 3 to 4 ($n = 6$), GCS 5 to 6 ($n = 11$), GCS 7 to 9 ($n = 9$), GCS 10 to 13 ($n = 7$), and GCS 14 to 15 ($n = 6$). All patients had GCS ≤ 8 within 48 hours after trauma. After arrival to NICU, the patients were then taken to surgery for placement of an intracranial ventricular catheter within 4 hours. The mean hospital stay was 18.9 days, and the hospital mortality was 15% (6/39).

The concentrations of vCSF total tau on days 2 to 3 post trauma correlated to morbidity and mortality at 1 year ($R^2 = 0.17$, $R = 0.42$, $p = 0.001$).

Nonsurvivors (GOSE 1) at 1-year follow-up had higher levels of vCSF total tau (8,500 pg/mL, IQR 7,638) on days 2 to 3 post trauma compared with survivors (GOSE 2 to 8), with levels of 682 pg/mL (IQR 1,155, $p < 0.001$) (figure 1). Patients with bad outcome (GOSE 1 to 4) had higher levels of vCSF total tau (2,580 pg/mL, IQR 7,443) vs those with good outcome (GOSE 5 to 8) (504 pg/mL, IQR 1,256) on days 2 to 3 ($p < 0.01$) (figure 2).

The vCSF total tau levels in NPH patients (reference group) were 677 pg/mL (IQR 308). When comparing these levels vs those of TBI patients, those succumbing (GOSE 1) had increased vCSF total tau levels at all sample intervals, days 2 to 3 ($p < 0.001$), days 4 to 6 ($p < 0.001$), and days 8 to 14 ($p < 0.001$).

TBI survivors (GOSE 2 to 8) had higher levels of vCSF total tau vs NPH patients on days 4 to 6 ($p < 0.05$) and days 8 to 14 ($p < 0.01$). Patients with poor outcome (GOSE 1 to 4) had increased levels of vCSF total tau at all sample intervals, days 2 to 3 ($p < 0.001$), days 4 to 6 ($p < 0.01$), and days 8 to 14 ($p < 0.001$) compared with patients with NPH. In patients with good outcome (GOSE 5 to 8), similar increases of vCSF total tau could be detected only on days 8 to 14 ($p < 0.001$).

One-year outcome assessed with GOSE demonstrated that of the 39 patients included, 51% (20/39) had a good outcome (GOSE 5 to 8), whereas 49% (19/39) had a bad

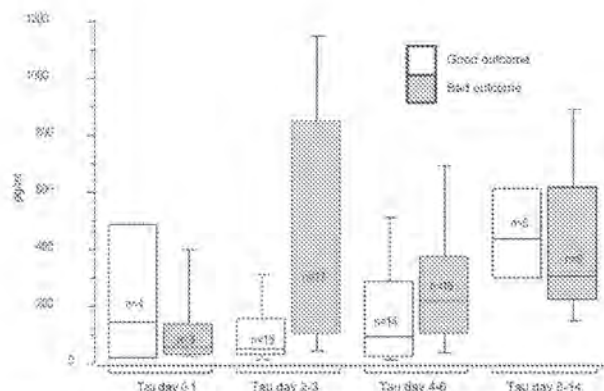


Figure 2. Tau levels days 0 to 14 in patients with traumatic brain injury related to 1-year good (Extended Glasgow Outcome Scale [GOSE] 5 to 8) vs bad (GOSE 1 to 4) outcome. ** $p < 0.01$.

outcome (GOSE 1 to 4). The 1-year mortality (GOSE 1) was 18% (7/39).

With ROC, we detected a sensitivity of 100% and a specificity of 81.5% for 1-year mortality at a vCSF total tau level above 2,126 pg/mL on days 2 to 3 (table). The calculated AUC was 0.934 (figure 3). The specificity, sensitivity, and LR+ of different cut-off levels for mortality of vCSF total tau are depicted in the table. Patients with bad outcome (GOSE 1 to 4) had a cut-off level of vCSF total tau of >702 pg/mL, with a sensitivity of 83.3% and a specificity of 69%, with a calculated AUC of 0.814 (figure 4).

No correlation could be found between NIHSS, investigating neurologic status 1 year post trauma, and initial vCSF total tau levels. Correspondingly, there was no correlation between initial vCSF total tau levels and ADL, assessed by Bartel Scale, 1 year post trauma.

Throughout the study, there were no detectable levels of total tau in serum.

Discussion. Using tau as a marker for axonal damage studying patients with severe TBI, we could verify that axonal damage is linked to 1-year morbidity (GOSE 2 to 4) as well as mortality (GOSE 1). We could also demonstrate a correlation between initially low vCSF total tau and good 1-year outcome (GOSE 5 to 8). The results indicate a cut-off level of vCSF total tau of >2,126 pg/mL related to 1-year

Table Calculated specificity and sensitivity and positive likelihood ratio (LR+) of different cut-off levels of ventricular CSF total tau on days 2 to 3 in patients with traumatic brain injury

Tau, pg/mL	Sensitivity	Specificity	100 - Specificity	LR+
11,060	28.6	96.3	3.7	7.71
8,500	57.1	96.3	3.7	15.4
4,040	71.4	92.6	7.4	9.6
3,760	71.4	88.9	11.1	6.4
2,938	85.7	85.2	14.8	5.8
2,126	100	81.5	18.5	5.4

LR+ is a calculated value of sensitivity (100 - specificity).

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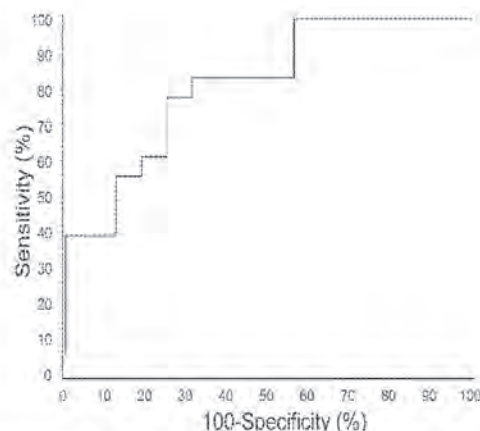


Figure 3. Receiver operator curve for 1-year mortality. Calculated sensitivity and specificity for tau levels in patients with traumatic brain injury on days 2 to 3.

mortality (GOSE 1), with a sensitivity of 100% and a specificity of 81.5%.

In the current study, vCSF was exclusively obtained for analysis. We have previously demonstrated a difference between vCSF and lumbar CSF (lCSF) total tau concentrations, with levels being higher in vCSF.¹¹ The way of obtaining CSF may thus be very important. This notion may not previously been appreciated as a mix of both vCSF and lCSF has been used in both reference and treatment groups in many investigations.

Thus, in a small ($n = 15$), retrospective study in TBI patients,¹² the authors presented similar vCSF tau levels as ours. They could, however, in contrast to our results, not detect a correlation between vCSF tau and in-house outcome. Their control group was a mixture of patients with dementia, headache, and NPH with analyses made on lCSF, which may explain their lower levels of lCSF tau compared with our homogeneous reference group of NPH patients,

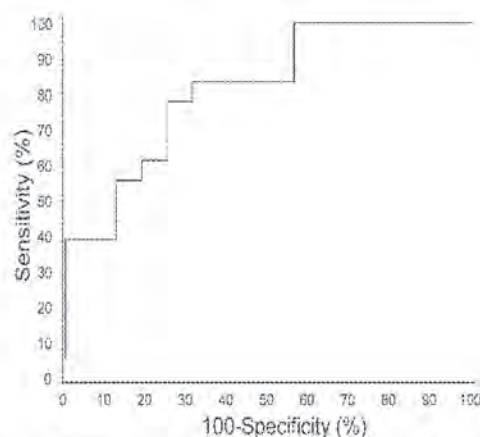


Figure 4. Receiver operator curve for 1-year bad (Extended Glasgow Outcome Scale [GOSE] score of 1 to 4) outcome. Calculated sensitivity and specificity for tau levels in patients with traumatic brain injury on days 2 to 3.

with total tau analyzed exclusively from vCSF. In a recent article about cleaved tau (c-tau) in TBI patients, the authors similarly did not state how CSF was collected.¹³

Tau protein is a small phosphoprotein found in the axonal compartments of neurons binding to microtubules promoting their stability and assembly.¹² Human brain tau protein has six isoforms, with molecular mass of approximately 48 to 67 kD on sodium dodecyl sulfate polyacrylamide gel electrophoresis.¹⁴ Increased levels of CSF tau are probably a sign of axonal injury and have been reported in Alzheimer disease (AD),^{15,16} Creutzfeldt-Jakob disease,¹⁷ Guillain-Barré syndrome,¹⁶ as well as in TBI.¹⁸ Axonal injury is a common feature of TBI damaging intracellular microtubules, and proteins like tau are then supposedly released into vCSF. Thus, speculatively increased levels of vCSF total tau may signal the development of an intracranial lesion, with higher levels of vCSF total tau correlating to poor outcome, results in accordance with the current investigation.

When analyzing, we could not find any detectable serum levels of total tau. We therefore present only total tau analyzed in vCSF. On the contrary, in a previous investigation, arterial blood levels of c-tau ranging from 5 to 12 ng/mL were detected.¹⁹

In 1999, using monoclonal antibodies developed by immunizing mice with tau proteins and screening hybridomas with CSF from brain trauma patients, one research group reported the presence of a set of 30- to 50-kD proteins in the CSF of brain trauma patients,²⁰ suggested to be c-tau, lacking the N- and C-terminal domains. These antibodies show poor reactivity with recombinant tau,²⁰ and no sequencing data on the proteins they recognize have been presented. The antibodies used in the current work recognize tau proteins of the expected size in CSF,²¹ similar to the molecular mass of CSF tau proteins reported by other groups.^{22,23} The relation between the tau proteins detected by our method and c-tau cannot be determined at present.

Tau is normal in lCSF of patients with NPH, indicating no or minor cortical degeneration in this disorder. In vCSF, total tau levels in patients with NPH levels have been estimated to 550 ng/L.²⁴ We have not found any investigations measuring vCSF total tau in healthy volunteers, but it does not seem pertinent to assume that volunteers may have even lower vCSF total tau values than patients with NPH.

In the current study, we have analyzed total tau, that is, including subforms. Other research groups have utilized various methods in quantifying tau. Groups from Cincinnati, OH, have measured c-tau in CSF in nanograms per milliliter, whereas more recent investigations, similar to the current one, analyze total tau measured in picograms per milliliter.^{13,14} c-tau cannot be found in CSF in NPH patients, whereas we and others measuring total tau also in NPH patients can detect this in both lCSF and vCSF.²⁵

Previous investigations in mice with closed head

injury have demonstrated an early rise in CSF tau within hours after TBI, followed by a decline within 24 to 48 hours.²⁶ This decline is then followed by a secondary increase in CSF tau levels on days 2 to 4. A new CSF tau decline is again noted followed by a third increase in CSF tau levels, peaking on day 10 to 14, returning to normal levels on day 43.¹²

Most of our patients were not admitted to our tertiary university hospital and received a ventricular catheter until 24 to 48 hours post trauma. One could question if the insertion of a ventricular catheter by itself would influence these levels. However, tau levels do not increase after insertion (patients get a catheter within 4 hours from admittance). The higher tau levels seen in this study are noted on day 2 to 3 independent of insertion of a ventricular catheter.

Thus, we could not obtain any really early vCSF tau samples for analysis. In our investigation, we could however confirm, from day 2 and on, the previously experimentally noted variations¹² in vCSF total tau levels in patients with bad outcome (GOSE 1 to 4) but not in patients with good outcome (GOSE 5 to 8). However, on days 8 to 14, there was a rise in vCSF total tau in all patients compared with those on days 0 to 1.

The initial vCSF total tau rise was very prominent in those dying (GOSE 1), with values more than 10-fold higher than in patients with good outcome (GOSE 5 to 8). All patients with lethal injury (GOSE 1) had vCSF total tau levels above 2,126 pg/L on days 2 to 3, that is, 100% sensitivity for death. We could also detect differences of vCSF total tau levels on days 2 to 3 between patients with bad outcome (GOSE 1 to 4) and those with good outcome (GOSE 5 to 8). The temporal variations in vCSF total tau levels during the first week, most prominent in patients with bleak 1-year outcome, may reflect a secondary CNS injury.¹⁷ In the current investigation, we detected increases in vCSF total tau on days 8 to 14 in all patients compared with the initial days post trauma.

A correlation between TBI and AD has been noted particularly.²⁷ The intracellular neuropathologic component of AD diagnosis is the presence of neurofibrillary tangles consisting of subunits of hyperphosphorylated tau.²⁸ AD is neuropathologically defined by the presence of both amyloid β -protein (A β) as well as tau, and novel research now links A β and neurofibrillary tangles containing tau. It seems that A β promotes caspase cleavage of tau. Caspase enzymes are connected to apoptosis, at least in experimental neonatal studies.²⁹ One can only speculate if the tau peaks noted by others and us are induced by caspase and represent the ongoing apoptosis noted after TBI.

Thus, in the current study, 1-year neurologic assessment was chosen. To capture even small differences in neurologic status, a battery of well-known tests were utilized. We used 1) GOSE, reflecting an overall measure of outcome; 2) NIHSS, evaluating

neurologic status; and 3) the Bartel Index, rating ADL. To further strengthen our outcome measurements, the assessment time (1 year) was very fixed, with variations only by days.

A limitation of the study is that of all included patients ($n = 55$), 16 were excluded as having an unstable ICP preventing collection of even small volumes of vCSF. Presumably these patients would have had a high vCSF total tau, and if so, these values would be in concert with the presented values. Unstable ICP also prevented occasional CSF samples in included patients. Further, patients were not transferred to our tertiary unit rapidly enough, rendering a late enclosure and vCSF collection until days 1 to 2.

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References

1. Fehlings LE, Hertz H, Mauritz W, et al. Nonspecific increase of systemic neuron-specific enolase after trauma: clinical and experimental findings. *Shock* 2005;24:119–123.
2. Raabe A, Grolms C, Sorge O, Zimmermann M, Seifert V. Serum S-100B protein in severe head injury. *Neurosurgery* 1999;45:477–483.
3. Nylen K, Ost M, Cserjok LZ, et al. Increased serum-GFAP in patients with severe traumatic brain injury is related to outcome. *J Neurol Sci* 2005;240:85–91.
4. Bennett M, O'Brien DP, Phillips JP, Farrell MA. Clinicopathologic observations in 100 consecutive patients with fatal head injury admitted to a neurosurgical unit. *Ir Med J* 1995;88:60–62.
5. Meythaler JM, Peduzzi JD, Eleftheriou E, Novack TA. Current concepts: diffuse axonal injury-associated traumatic brain injury. *Arch Phys Med Rehabil* 2001;82:1461–1471.
6. Goedert M. Tau protein and the neurofibrillary pathology of Alzheimer's disease. *Trends Neurosci* 1993;16:460–465.
7. Grande PO, Asgeirsson B, Nordstrom CH. Volume-targeted therapy of increased intracranial pressure: the Lund concept unifies surgical and non-surgical treatments. *Acta Anaesthesiol Scand* 2002;46:929–941.
8. Tullberg M, Månsson JE, Fredman P, et al. CSF sulfatide distinguishes between normal pressure hydrocephalus and subcortical arteriosclerotic encephalopathy. *J Neurol Neurosurg Psychiatry* 2000;69:74–81.
9. Blennow K, Fredman P. Detection of cerebrospinal fluid leakage by isoelectric focusing on polyacrylamide gels with silver staining using the PhastSystem. *Acta Neurochir (Wien)* 1995;136:135–139.
10. Wilson JT, Pettigrew LE, Teasdale GM. Structured interviews for the Glasgow Outcome Scale and the Extended Glasgow Outcome Scale: guidelines for their use. *J Neurotrauma* 1998;15:573–585.
11. Blennow K, Nollgard B. Amyloid beta 1-42 and tau in cerebrospinal fluid after severe traumatic brain injury. *Neurology* 2004;62:160.
12. Franz G, Beer R, Kampfl A, et al. Amyloid beta 1-42 and tau in cerebrospinal fluid after severe traumatic brain injury. *Neurology* 2003;60:1457–1461.
13. Chatfield DA, Zemlan FP, Day DM, Menon DK. Discordant temporal patterns of S100beta and cleaved tau protein elevation after head injury: a pilot study. *Br J Neurosurg* 2002;16:471–476.
14. Goedert M, Spillantini MG, Cairns NJ, Crowther RA. Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. *Neuron* 1992;8:159–168.
15. Hulstaert F, Blennow K, Ivanov A, et al. Improved discrimination of AD patients using beta-amyloid(1-42) and tau levels in CSF. *Neurology* 1999;52:1555–1562.
16. Vandermeeren M, Mercken M, Vannochelen E, et al. Detection of tau proteins in normal and Alzheimer's disease cerebrospinal fluid with a sensitive sandwich enzyme-linked immunosorbent assay. *J Neurochem* 1993;61:1828–1834.
17. Otto M, Wiltfang J, Tumani H, et al. Elevated levels of tau-protein in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. *Neurosci Lett* 1997;225:210–212.
18. Kay AD, Petzold A, Kerr M, Keir G, Thompson E, Nicoll JA. Alterations in cerebrospinal fluid apolipoprotein E and amyloid beta-protein after traumatic brain injury. *J Neurotrauma* 2003;20:943–952.
19. Zemlan FP, Jauch EC, Mulchahey JJ, et al. C-tau biomarker of neuronal damage in severe brain injured patients: association with elevated intracranial pressure and clinical outcome. *Brain Res* 2002;947:131–139.
20. Zemlan FP, Rosenberg WS, Luebbe PA, et al. Quantification of axonal damage in traumatic brain injury: affinity purification and characterization of cerebrospinal fluid tau proteins. *J Neurochem* 1999;72:741–750.
21. Sjögren M, Davidsson P, Tullberg M, et al. Both total and phosphorylated tau are increased in Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 2001;70:624–630.
22. Arai H, Terajima M, Miura M, et al. Tau in cerebrospinal fluid: a potential diagnostic marker in Alzheimer's disease. *Ann Neurol* 1995;38:649–652.
23. Vigo-Pelfrey C, Seubert P, Barbour R, et al. Elevation of microtubule-associated protein tau in the cerebrospinal fluid of patients with Alzheimer's disease. *Neurology* 1995;45:788–793.
24. Tullberg M, Blennow K, Månsson JE, Fredman P, Tisel M, Wikkelso C. Ventricular CSF neurofilament protein levels decrease in parallel with white matter pathology after shunt surgery in normal pressure hydrocephalus. *Eur J Neurol* (in press).
25. Tisel M, Tullberg M, Månsson JE, Fredman P, Blennow K, Wikkelso C. Differences in cerebrospinal fluid dynamics do not affect the levels of biochemical markers in ventricular CSF from patients with aqueductal stenosis and idiopathic normal pressure hydrocephalus. *Eur J Neurol* 2004;11:17–23.
26. Genis L, Chen Y, Shohami E, Michaelson DM. Tau hyperphosphorylation in apolipoprotein E-deficient and control mice after closed head injury. *J Neurosci Res* 2000;60:559–564.
27. Corkin S, Rosen TJ, Sullivan EV, Clegg RA. Penetrating head injury in young adulthood exacerbates cognitive decline in later years. *J Neurosci* 1989;9:3876–3883.
28. Selkoe DJ. Defining molecular targets to prevent Alzheimer disease. *Arch Neurol* 2005;62:192–195.
29. Blomgren K, Zhu C, Wang X, et al. Synergistic activation of caspase-3 by m-calpain after neonatal hypoxia-ischemia: a mechanism of "pathological apoptosis"? *J Biol Chem* 2001;276:10191–10198.

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Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury

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APPENDIX 3

τ Proteins in Serum Predict Outcome After Severe Traumatic Brain Injury

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Background. The identification of reliable outcome predictors after traumatic brain injury (TBI) is crucial. The objective of our study was to investigate the role of τ protein as a serum marker of TBI.

Methods. Thirty-four patients with severe TBI (Glasgow Coma Scale [GCS] score at admission ≤ 8) were considered. The τ protein level in the blood samples obtained at the time of admission was measured. The outcome was assessed by using the Glasgow Outcome Scale (GOS) at 6 mo post-injury. Demographic, clinical, and laboratory variables were analyzed to study their effect on the outcome.

Results. τ Protein levels were higher in the poor outcome group (436.2 ± 473.6 pg/mL) than in the good outcome group (51.6 ± 81.5 pg/mL) ($P < 0.0001$). Univariate analyses demonstrated that poor outcome was significantly associated with a poor GCS score ($P = 0.001$), higher serum τ protein levels ($P < 0.001$), abnormal pupil light reflex ($P = 0.013$), and basal cistern compression on computed tomogram (CT) ($P = 0.026$). Multivariate analyses revealed that a poor GCS score ($P = 0.049$) and higher serum τ protein levels ($P = 0.043$) were independent prognostic factors for poor outcome. The receiver-operating characteristic (ROC) curve demonstrated that a τ protein level ≥ 114.5 pg/mL yielded 88% sensitivity and 94% specificity for predicting a poor outcome.

Conclusions. These results suggest that in addition to GCS, serum τ protein levels may serve as indicators for the prediction of outcome following severe TBI.

However, it should be viewed with caution because of the small sample size and wide standard deviations. © 2010 Elsevier Inc. All rights reserved.

Key Words: tau protein; outcome; traumatic brain injury; ELISA.

INTRODUCTION

Traumatic brain injury (TBI) is the leading cause of death and disabilities among all traumas [1, 2]. The identification of reliable indicators of outcome following TBI is crucial for the determination of appropriate clinical management and for predicting clinical outcome. Reliable prediction of outcome following severe TBI, especially in the early stages, has still not been achieved [3]. Although clinical characteristics such as the Glasgow Coma Scale (GCS) score and imaging studies are well established, there are still some concerns regarding these tools [4]. It would be beneficial to supplement these methods of investigation with some chemical biomarkers for TBI [4–11].

τ Protein is a microtubule-associated protein that is primarily localized in neuronal cells. τ Binds to axonal microtubules and forms axonal microtubule bundles. These bundles are important structural elements in the axonal cytoskeleton. The level of τ proteins in the serum and cerebrospinal fluid (CSF) can be determined, and it is released from the central nervous system secondarily because of TBI [8–11]. Evidence indicates that τ protein levels are elevated in the CSF [8, 9] and serum following TBI [10, 11]. However, data

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on serum τ protein levels in patients with severe TBI are sparse. The predictive value of serum τ protein levels in determining outcome in cases of severe TBI have not been investigated thoroughly. The aim of the present study is to clarify whether τ protein levels could predict 6-mo outcomes in patients with severe TBI.

MATERIALS AND METHODS

Patients

This study was approved by the local ethics committee of the E-Da Hospital in Taiwan. Prior to enrollment, written informed consent was obtained from each patient's next of kin because all the eligible patients were unconscious. Thirty-four patients, aged 18 y or more, with severe closed head injury who were admitted to the neurosurgical intensive care unit (NICU) at E-Da Hospital, Kaohsiung, Taiwan, within 6 h of injury were enrolled in this study between April 2006 and January 2007. Severe TBI was defined as a Glasgow Coma Scale (GCS) score of 8 or less following nonsurgical resuscitation within the first 6 h of injury. Computed tomography (CT) was performed immediately after resuscitation. Following resuscitation and evacuation of mass lesions, if indicated, all patients were admitted to NICU and managed according to a protocol that was consistent with the international guidelines for severe TBI [12]. Ten healthy volunteers (6 men and 4 women) served as the control group.

Exclusion Criteria

The exclusion criteria were as follows: age under 18 y, unavailability of blood sample, absence of informed consent, and absence of outcome data of 6-mo follow-up. Patients with neurologic disabilities (head injury, cerebral infarction, and hemorrhage) prior to the injury were also excluded. The following patients were also excluded: patients who were brought to the hospital more than 6 h after injury, prolonged cardiac arrest at the scene of the accident, or high cervical spinal cord injury, and those who had died from uncontrollable hemorrhage or multiple life-threatening associated injuries.

Analysis of Serum τ Protein

Venous blood samples were obtained at the time of hospital admission. Samples were drawn into 10-mL serum separator tubes and centrifuged at 2500 rpm for 15 min after collection. The serum was then stored at -70°C until analysis. The serum τ protein levels were measured using a sandwich enzyme linked-immuno-sorbent assay (ELISA) kit (BioSource, Camarillo, CA). The minimum detectable dose of τ protein is 12 pg/mL [13]. The dilution curve of the serum samples was parallel to the standard dilution curve.

Prognostic Factors

To study prognostic factors that determine outcome for each patient, the following data were collected for each patient at the time of admission: age, gender, GCS score, cause of injury, head abbreviated injury score (AIS) [14], pupil abnormality and light reflex, CT finding, intracranial pressure (ICP), systemic hypotension, τ protein level, and blood ethanol level. Systemic hypotension was defined as systolic blood pressure ≤ 90 mmHg at the time of admission. A midline shift ≥ 5 mm and the obliteration of basal cisterns as observed on a CT scan were considered as outcome predictors [15]. The AIS is an anatomic scoring system that ranks injury severity on a scale of 1 to 6; a score of 3 represents serious; 4, severe; 5, critical; and 6, fatal.

Outcome Measurement

Outcome was categorized according to the Glasgow Outcome Scale [16] as asymptomatic, mild disability, moderate disability, major disability, vegetative status, and death. For the analysis, patients were divided into a good-outcome group, with patients who were functionally independent (first three categories), and a poor-outcome group, with patients who were functionally dependent or worse (last three categories). The outcome for the survivors was determined at 6 mo after TBI. Some patients were followed-up by the outpatient department after discharge, while others were interviewed over telephone to identify the neurological outcome.

Statistical Analysis

Variables were initially analyzed by univariate analysis. Continuous variables (for example, age) were compared using the Mann-Whitney *U* test. Categorical variables (for example, pupil abnormality) were analyzed either by χ^2 analysis or Fisher's exact test. Multivariate analysis with logistic regression was then carried out to determine the factors that were also prognostically important for the outcome as defined above. Receiver-operating characteristic (ROC) curve was used to determine the τ protein level that would yield the best sensitivity and specificity for predicting outcome. Positive likelihood ratio was calculated by dividing the sensitivity by (100-specificity). The likelihood ratio may be used to determine clinically relevant cut-off values. Statistical tests were conducted by using SPSS 12.0 (SPSS Inc, Chicago, IL). All *P* values are two-sided, and *P* < 0.05 was considered statistically significant.

RESULTS

Figure 1 shows a flow diagram of how patients were included or excluded in the study. The demographic and injury characteristics of patients are shown in Table 1. The τ protein levels (mean $\pm$ SD) were 8.1 ± 20.8 pg/mL (range, 0 to 67) in the control group (*n* = 10), undetectable (< 12 pg/mL) in 9 of the 10 volunteers, and 243.9 ± 387.4 pg/mL (range, 0 to 1882) in the patient group (*n* = 34). Significant differences were found between the control and patient groups (*P* < 0.0001). The τ protein levels in the good-outcome group were 51.6 ± 81.5 pg/mL (range, 0 to 342) (*n* = 17) and 436.2 ± 473.6 pg/mL (range, 41 to 1882) in the poor-outcome group (*n* = 17). The τ protein levels were higher in the poor-outcome group than in the good-outcome group (Table 2, Fig. 2).

Univariate analyses revealed that poor outcome at 6 mo was significantly associated with the following features: poor GCS score (*P* = 0.001), high serum τ level (*P* < 0.001), abnormal pupil light reflex (*P* = 0.013), and basal cistern compression on CT scan (*P* = 0.026). Multivariate logistic regression analyses revealed that a poor GCS score (*P* = 0.049) and high serum τ levels (*P* = 0.043) were the independent prognostic factors for a poor outcome (Table 3).

The range of serum τ protein levels was broad (0–1882 pg/mL) in the present study, and the clinical utility of this biomarker was limited. An ROC evaluating the ability of serum τ protein level to predict a poor

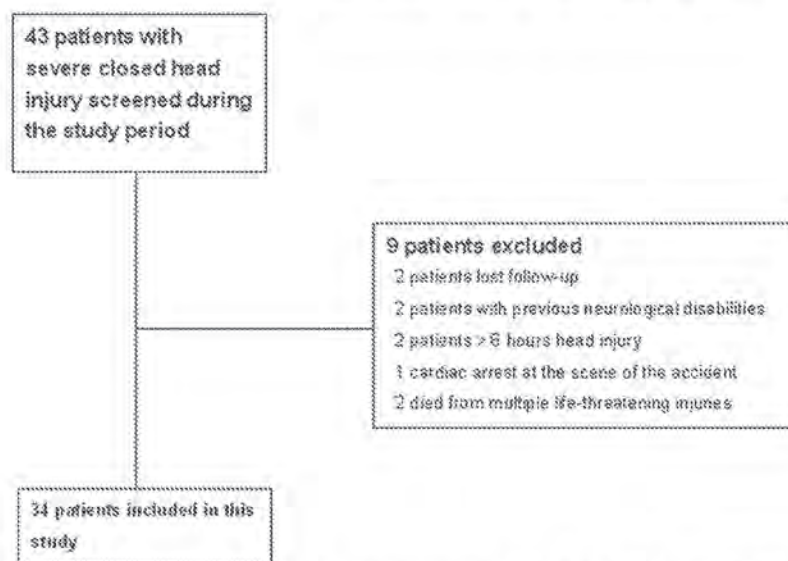


FIG. 1. Flow diagram demonstrating how patients were included or excluded in the study.

outcome showed an area under the curve of 0.939 with 88% sensitivity and 94% specificity at a serum τ protein level of 114.5 pg/mL (Fig. 3). As shown in Table 4, in the poor-outcome group, 15 patients (88.2%) had τ protein

levels higher than 114.5 pg/mL, and only two patients had τ level lower than 114.5 pg/mL. Sixteen patients (94.1%) in the good-outcome group had τ protein levels lower than 114.5 pg/mL. The positive predictive value was 93.8%, and the negative predictive value was 88.9%.

TABLE 1
Demographics of 34 Patients with Severe Traumatic Brain Injury

Age, y (range)	40.7 $\pm$ 17.9 (18–77)
Gender, n (%)	
Male	27 (79)
Female	7 (21)
GCS, n (%)	
3–4	5 (15)
5–6	8 (23)
7–8	21 (62)
Abbreviated injury score, n (%)	
3	6 (18)
4	10 (29)
5	18 (53)
Serum τ level in pg/mL, mean $\pm$ SD	243.9 $\pm$ 387.4 (0–1882)
Cause of injury, n (%)	
Fall	28 (82)
Motor vehicle accident	4 (12)
Assault	2 (6)
Main lesion (%)	
Epidural hematoma	3 (9)
Subdural hematoma	16 (47)
Subarachnoid hematoma	7 (21)
Intracerebral hematoma	8 (23)
Craniotomy, n (%)	20 (59)
ICP monitor, n (%)	19 (56)
Outcome (n = 34), n (%)	
Good outcome	17 (50)
Poor outcome	17 (50)

ICP = intracranial pressure; GCS = Glasgow coma scale.

DISCUSSION

The serum τ levels in severe TBI cases (243.9 $\pm$ 387.4 pg/mL) were higher than those in healthy volunteers (8.1 $\pm$ 20.8 pg/mL) in this study. The findings support the theory that serum τ protein levels could increase after TBI [10, 11]. The present study also demonstrated that serum τ levels were significantly higher in the TBI patients with poor outcomes and could be used as an independent prognostic factor for severe TBI. Multivariate analysis revealed that the GCS score and the serum τ protein level was significantly associated with clinical outcome. ICP, age, pupil light reflex, AIS, systemic hypotension, midline shift on CT scan, and cistern compression on CT scan were not associated with outcome in our study, unlike in previous reports [14, 15, 17], which could be due to the smaller sample size of the present study.

τ Protein is a small phosphor protein found in the axonal compartments of neurons that bind with microtubules, promoting their stability and assembly [8, 9]. The level of τ protein in the CSF and serum can be determined, and it is released from neurons of the central nervous system following TBI [8–11]. The rise in τ protein levels in the CSF was very prominent (more than

TABLE 2
Comparisons of Clinical and Laboratory Features Between the Good and Poor Outcome Groups of Severe Traumatic Brain Injury Patients

	Good outcome <i>n</i> = 17	Poor outcome <i>n</i> = 17	<i>P</i> Value
Age, <i>y</i> (mean $\pm$ SD)	36.9 $\pm$ 19.3	44.4 $\pm$ 16.2	0.131
Gender, <i>n</i> (%)			1.0
Male	13 (48)	14 (52)	
Female	4 (57)	3 (43)	
Glasgow coma score, <i>n</i> (%)	7.6 $\pm$ 0.6	5.4 $\pm$ 1.9	0.001
Causes of head injury, <i>n</i> (%)			1.0
MVA	14 (50)	14 (50)	
Fall	2 (50)	2 (50)	
Assault	1 (50)	1 (50)	
Pupil light reflex, <i>n</i> (%)			0.013
Normal	14 (70)	6 (30)	
Absent or sluggish in either or both	3 (21)	11 (79)	
Intracranial pressure > 20 mmHg ^a , <i>n</i> (%)	1 (10)	9 (90)	0.057
Midline shift > 5 mm on CT, <i>n</i> (%)	8 (44)	10 (56)	0.492
Basal cistern space on CT, <i>n</i> (%)			0.026
Normal	9 (82)	2 (18)	
Compressed or absent	8 (35)	15 (65)	
τ Protein level, pg/mL (mean $\pm$ SD)	51.6 $\pm$ 31.5	436.2 $\pm$ 473.6	<0.001
Underwent craniotomy, <i>n</i> (%)	9 (45)	11 (55)	0.486
Abbreviated injury score (mean $\pm$ SD)	4.2 $\pm$ 0.8	4.5 $\pm$ 0.7	0.218
Alcohol screen positive, <i>n</i> (%)	5 (63)	3 (37)	0.688

MVA = motor vehicle accident; CT = computed tomography.

^aData available 19 cases.

1000-fold) in severe TBI patients [9]. High τ levels in the CSF were associated with poor outcome in some studies and were considered to be useful as an outcome predictor [8, 9].

Clinical research on the effects of TBI on serum τ protein levels is limited [10, 11]. Kavalci and colleagues [10] reported that serum τ levels were increased in patients with mild head injury and intracranial lesions; however, the rise was not statistically significant. Buhit

and colleagues [11] reported that the serum τ levels in patients with mild head injury were relatively higher than those in controls; however, the differences were not statistically significant. This suggests that serum τ protein has limited prognostic value for minor head injury.

Our data demonstrates that patients with epidural hematomas have lower serum τ levels. There were three patients with epidural hematoma in our study, and their τ levels were less than 12 pg/mL. Perhaps patients with epidural hematoma had mild parenchymal brain injury and lower τ levels.

The serum τ protein levels in severe TBI patients have not been well investigated. To the authors' knowledge, the current study is the first to measure and evaluate the serum τ levels in severe TBI patients. In the present study, the τ levels increased predominantly in the poor-outcome patients and were indicated to be potentially useful in predicting 6-mo outcomes following severe TBI. The results could be explained by the fact that the subjects in the present study, contrary to those in previous studies [10, 11], had uniformly more severe TBI.

The range of serum τ protein levels was very broad (0–1882 pg/mL) in the present study, and the clinical utility of this biomarker was limited. An ROC evaluating the ability of serum τ protein levels to predict poor outcome showed an area under the curve of 0.939

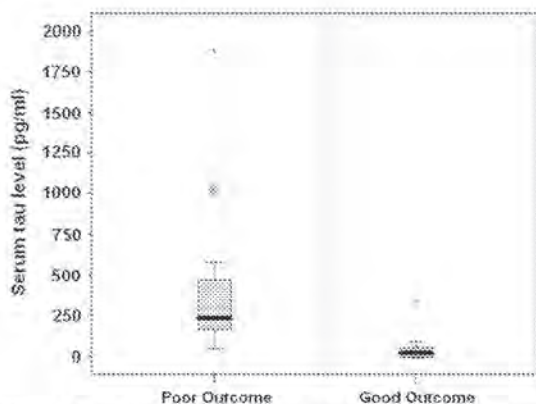


FIG. 2. Boxplot showing serum τ protein levels in both outcome groups following severe traumatic brain injury. There was a significant difference between good and poor outcome groups ($P < 0.0001$; Mann-Whitney *U* test). (Color version of figure is available online.)

TABLE 3
Results of Multivariate Logistic Regression Analyses of Factors in Predictive of Poor Outcome in Severe Traumatic Brain Injury Patients

Variable	Coefficient	Standard error	95% CI	P value
τ Protein level, pg/mL	-0.015	0.008	0.970–0.999	0.043
GCS	1.574	.799	1.009–23.081	0.049
Abnormal pupil light reflex	-2.850	2.296	0.001–5.211	0.215
basal cistern compress on CT	-2.448	1.979	0.002–4.180	0.216

GCS = glasgow coma scale; CT = computed tomography.

with 88% sensitivity and 94% specificity at a serum τ protein level of 114.5 pg/mL. At the serum τ level of 114.5 pg/mL, the positive predictive value for poor outcome was 93.8%, and the negative predictive value was 88.9%. Another advantage of serum τ protein is that it can be measured by less invasive methods than those used for determining the CSF levels.

Some research studies reported that cleaved τ (c- τ) could be detected in the CSF and serum of TBI patients [18–20]. C- τ could reflect the severity of TBI [18] and predict intracranial lesions [20]. C- τ , reported by Zemlan and colleagues [18–20], is a truncated τ protein (30 to 50 Kd) that is shorter than the full-length τ protein (48 to 68 Kd) and lacks the N- and C-terminal domains. Because of different measurement methods, the relation between the τ proteins detected by our methods [8–11] and c- τ by other groups [18–21] cannot be determined at present.

The present study has several limitations. We had examined many well-documented factors (such as age,

ICP, and systemic hypotension) affecting clinical outcome following severe TBI; however, some factors had little utility for predicting clinical outcome. This could be due to the relatively small sample size of the present study. We did not examine the τ levels in the CSF because CSF samples could not be obtained using our ICP devices. Rather than using external ventriculostomy drainage, most ICP devices used in our patients were subdural or intraparenchymal microsensors [22]. The correlation between the CSF τ levels and serum τ levels was unclear. Two patients were lost to follow-up. This is an important limitation because patients with good outcome are more likely to be lost to follow-up. This may bias the population being studied. Furthermore, we did not obtain serial blood samples or standardize the time of blood sampling for all patients, which may have resulted in the observed variability of τ protein levels.

What time will τ protein be released to blood after TBI? To date, the progression of τ protein levels in TBI patients has rarely been reported. CSF τ levels were highest on days 2 to 3 in one study [8]; however, no time course of human serum τ levels following TBI were reported. The time course of serum τ protein level was reported in one rat TBI study [23] and the highest level was found 6 h following injury. The integrity of brain blood barrier (BBB) plays a role in serum and CSF τ level [23]. After BBB re-established following TBI, the τ levels fell.

Although the results demonstrate that serum τ protein levels and GCS are most important outcome predictors, these results should be viewed with caution because of the relatively small sample size of the

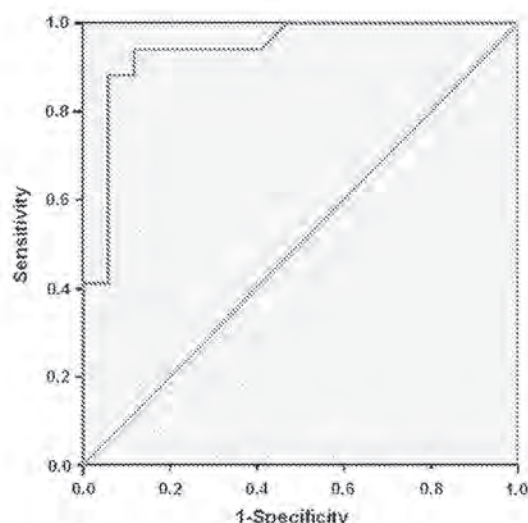


FIG. 3. Receiver operating characteristic curve for serum τ protein level for poor outcome at 6 mo following severe traumatic brain injury. The area under the curve was 0.939 (95% CI 0.856 to 1.022; $P < 0.001$). (Color version of figure is available online.)

TABLE 4
Patient Classification Based on the Receiver Operating Characteristic (ROC) Curve Results of the Serum τ Protein Level

	Good outcome <i>n</i> (%)	Poor outcome <i>n</i> (%)
$\tau < 114.5$ pg/mL	16 (94.1)	2 (11.1)
$\tau \geq 114.5$ pg/mL	1 (5.9)	15 (88.2)

present study. Future studies should include a more extensive examination of the interrelationship of τ protein levels and those outcome predictors that would require a larger cohort of TBI patients.

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REFERENCES

1. Chiu WT, Huang SJ, Tsai SH, et al. The impact of time, legislation, and geography on the epidemiology of traumatic brain injury. *J Clin Neurosci* 2007;14:930.
2. Lin JW, Tsai SH, Tsai WC, et al. Survey of traumatic intracranial hemorrhage in Taiwan. *Surg Neurol* 2006;66:S20.
3. Marion DW. Outcome from severe head injury. In: Narayan RK, Wilberger E, Povlishock JT, Eds. *Neurotrauma*. New York: McGraw Hill, 1996:767.
4. Woertgen C, Rothoerl R, Metz C, et al. Comparison of clinical, radiologic, and serum marker as prognostic factors after severe head injury. *J Trauma* 1999;47:1126.
5. Raabe A, Grolms C, Sorge O, et al. Serum S-100B protein in severe head injury. *Neurosurgery* 1999;45:477.
6. Korfiatis S, Stranjalis G, Boviatis E, et al. Serum S-100B protein monitoring in patients with severe traumatic brain injury. *Intensive Care Med* 2007;33:255.
7. Vos PE, Lamers KJ, Hendriks JC, et al. Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury. *Neurology* 2004;62:1303.
8. Ost M, Nylén K, Csajbok L, et al. Initial CSF total τ correlates with 1-year outcome in patients with traumatic brain injury. *Neurology* 2006;67:1600.
9. Franz G, Beer R, Kampf A, et al. Amyloid beta 1-42 and τ in cerebrospinal fluid after severe traumatic brain injury. *Neurology* 2003;60:1457.
10. Kavaleci C, Pekdemir M, Durukan P, et al. The value of serum τ protein for the diagnosis of intracranial injury in minor head trauma. *Am J Emerg Med* 2007;25:391.
11. Bulut M, Koksai O, Dogan S, et al. τ Protein as a serum marker of brain damage in mild traumatic brain injury: Preliminary results. *Adv Ther* 2006;23:12.
12. The Brain Trauma Foundation. American Association of Neurological Surgeons, Joint Section on Neurotrauma and Critical Care. Management and prognosis of severe traumatic brain injury. *J Neurotrauma* 2000;17:449.
13. Human T(total) Biosource Immunoassay Kit Catalog #KHB0042/KHB0041. Biosite website available at: <http://www.biosource.com/content/catalogContent/tot3/moreinfo/KHO6631%20pr328%20revA1%20mar2706%20Hu%20Tau%20ipT181b.pdf>
14. Foreman BP, Caesar RR, Parks J, et al. Usefulness of the abbreviated injury score and the injury severity score in comparison to the Glasgow Coma Scale in predicting outcome after traumatic brain injury. *J Trauma* 2007;62:946.
15. Schreiber MA, Anki N, Scott BG, et al. Determinants of mortality in patients with severe blunt head injury. *Arch Surg* 2002;137:285.
16. Jennett B, Bond M. Assessment of outcome after severe brain damage. *Lancet* 1975;1:469.
17. Tsai MD, Chiu WT, Lin JW, et al. Current experiences in the use of the severe head-injury guidelines in Taiwan. *Surg Neurol* 2006;66:S3.
18. Zemlan FP, Jauch EC, Mulchahey JJ, et al. C- τ biomarker of neuronal damage in severe brain injured patients: Association with elevated intracranial pressure and clinical outcome. *Brain Res* 2002;947:131.
19. Zemlan FP, Mulchahey JJ, Gudelsky GA. Quantification and localization of kainic acid-induced neurotoxicity employing a new biomarker of cell death: Cleaved microtubule-associated protein- τ (C- τ). *Neuroscience* 2003;121:399.
20. Shaw GJ, Jauch EC, Zemlan FP. Serum cleaved τ protein levels and clinical outcome in adult patients with closed head injury. *Ann Emerg Med* 2002;39:254.
21. Johnson GVW, Seubert P, Cox TM, et al. The τ protein in human cerebrospinal fluid in Alzheimer's disease consists of proteolytically derived fragments. *J Neurochem* 1997;68:430.
22. Huang SJ, Hong WC, Han YY, et al. Clinical outcome of severe head injury in different protocol-driven therapies. *J Clin Neurosci* 2007;14:449.
23. Gabbita SP, Scheff SW, Menard RM, et al. Cleaved- τ : A biomarker of neuronal damage after traumatic brain injury. *J Neurotrauma* 2005;22:83.

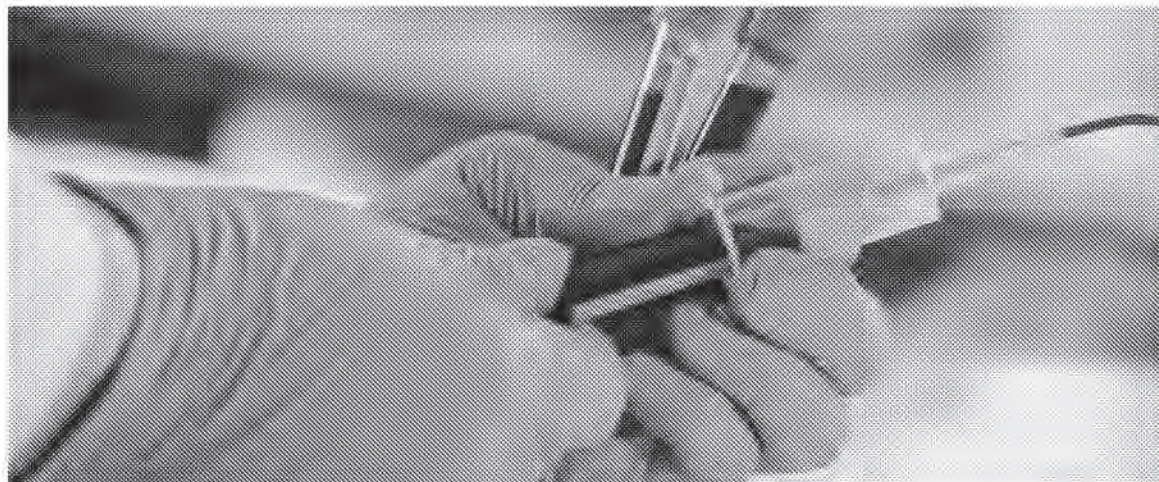
APPENDIX 4

April 19, 2021

A new market beckons for Alzheimer's blood tests



Elizabeth Cairns



If drugs acting on amyloid and tau can defy the sector's history of failure, the testing landscape will change dramatically.

Success in clinical trials of drugs for Alzheimer's disease seems to hinge in large part on picking the right patients. Recent positive – just – data on Lilly's project donanemab came from a trial that excluded patients with high tau levels, and dona's ongoing pivotal trial is using similar enrolment criteria.

Blood tests for tau are thus already in demand, and several groups are developing assays for this and other Alzheimer's biomarkers for clinical trial "enrichment". But test developers are looking beyond this niche. Blood tests for tau and even amyloid could come to be used to diagnose Alzheimer's; should dona, or Biogen's anti-amyloid therapy aducanumab, reach market, demand for this kind of testing could explode.

Lilly has developed an in-house test capable of picking up a variant of the protein called phospho-tau217 (p-tau217) shed by the brain into the blood. The company uses this in combination with PET imaging to stratify patients into low, medium and high tau groups for its trials of dona. In [Trailblazer Alz-2](#), the MAB's pivotal study, the best results are expected from the low-tau group ([Lilly moves to stack the deck for donanemab](#), March 16, 2021).

Cheaper and less invasive

But these assays have potential beyond aiding drug development. Last year [data published in jama](#) suggested that Lilly's test could tell patients with Alzheimer's apart from those with other neurodegenerative diseases, and its performance was not significantly different from cerebrospinal fluid or PET-based testing.

That said, the test is not sensitive enough on its own to quantify the amount of tau in patients' brains.

"We can use the blood to find people who have tau in the brain, kind of a yes/no," says John Sims, senior medical director of neurodegeneration at Lilly. The precision on just how much tau is present is not possible yet, he says, "and I don't know whether it will be".

Consequently in the diagnostic context tau tests could be used as a first pass, with those with a positive result being sent for confirmation with PET.

"How [this] will fit into the diagnostic armamentarium is still yet to be determined," says Mr Sims. "Certainly at minimum it could sit as an initial cheaper step for ensuring that someone has maybe the pathology, if they're

coming in complaining of symptoms."

The diagnostics group Quanterix also has a tau test. It launched an assay for a different variant of the protein, p-tau181, last year as a way to aid research into Alzheimer's and to help enrol the right patients into drug trials. This too has shown correlation with CSF and PET scan testing for Alzheimer's pathology.

Quanterix's product is therefore comparable to tests that require either a spinal tap or lengthy imaging procedures, which are "probably 500 times more expensive and much more invasive," says the group's chief executive, Kevin Hrusovsky.

He adds that the test is "selling off the charts" at the moment – but expects further growth should a tau-based treatment for Alzheimer's succeed.

"There's not a lot of people in the world today that would want to know, 'hey, your biomarker signature is telling us that you've got Alzheimer's, and you're probably going to get dementia 15 years from now'," Mr Hrusovsky says. "That's why in the area of Alzheimer's all of the focus right now is getting the drug across the goal line."

Mr Sims agrees, saying that Lilly has always seen the need for tau blood tests increasing once a therapeutic option is available.

All eyes on the tau pipeline, then. The most advanced candidate is Taurx Pharmaceuticals' tau aggregation inhibitor LMTX, whose Lucidity phase 3 trial is set to conclude at the end of this year. This study does not appear to be stratifying patients by tau levels; its record on clinicaltrials.gov lists the presence of amyloid as an inclusion criterion.

Lilly's Trailblazer Alz-2 study of donanemab, the most advanced trial that does screen enrollees for tau, will report in 2023.

Amyloid

There is of course a vitally important crunch point fast approaching for a different Alzheimer's biomarker. The FDA will pronounce on aducanumab's approvability in June, and the decision will either provide something approaching vindication for the amyloid hypothesis or do it an injury from which it might never recover.

There is in fact a blood test for beta-amyloid already on sale in the US. C2N Diagnostics launched PrecivityAD under a Clia certification last October, in much the same settings as tau tests: to help clinicians diagnose Alzheimer's in patients experiencing early memory complaints, and to screen patients for drug trials. Its list price is \$1,250, but C2N has a financial assistance programme that can lower the cost to between \$25 and \$400 for eligible patients.

C2N's chief executive, Joel Braunstein, says that despite the lack of active anti-amyloid drugs demand for PrecivityAD is strong.

"Some people would say, I'm not sure I want to know, and other people are very enthusiastic about having access to this information even in the absence of the disease-modifying therapy," he says. "There's a movement of people who want to be very proactive with their health, and are interested in being more informed."

If the test is positive, the patients can change their lifestyles in ways that have been proven to slow the progression of Alzheimer's, doing more physical exercise and taking part in cognitive and memory support training, for example.

Still, there is a limit to what can be achieved by these kinds of interventions. Availability of an anti-amyloid drug would change the situation.

"Certainly, if a disease-modifying therapy becomes available, there will be a considerable interest in having additional information to help get a precise diagnosis," Mr Braunstein says. "The less invasive nature of a blood draw will certainly be helpful for supporting an infrastructure that's going to presumably have a significant need to identify people with early pathology."

If – and it is of course a big if – anti-amyloid and anti-tau MABs make it to market, the setting for blood tests for these biomarkers could shift again.

Jeff Dage, distinguished medical fellow at Lilly, says that some research has suggested that Lilly's tau test can be used to screen people before symptom onset to identify those with tau pathology who are likely to develop Alzheimer's.

Quanterix's Mr Hrusovsky envisions annual or even quarterly screening programmes for healthy people, organised via insurers or other payer groups, as a potential use of the assays. "so that you can see the earliest cascade of amyloidosis or tauopathy".

This might be a stretch. But it is clear that the utility of blood tests for Alzheimer's markers – and to some extent the fate of the companies developing them – is tied closely to the success or otherwise of drugs like aducanumab and donanemab.

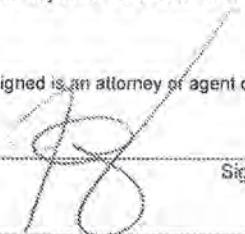
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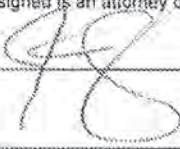
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For: METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY	
The applicant, <u>Quanterix Corporation</u> , owner of <u>100</u> percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending <u>reference</u> Application Number <u>14/111,331</u> , filed, <u>December 18, 2013</u> , as the term of any patent granted on said <u>reference</u> application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending <u>reference</u> application. The applicant hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the <u>reference</u> application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.	
In making the above disclaimer, the applicant does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said <u>reference</u> application, "as the term of any patent granted on said <u>reference</u> application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending <u>reference</u> application," in the event that: any such patent granted on the pending <u>reference</u> application expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.	
Check either box 1 or 2 below, if appropriate.	
1 ☒ The undersigned is the applicant. If the applicant is an assignee, the undersigned is authorized to act on behalf of the assignee.	
I hereby acknowledge that any willful false statements made are punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.	
2. ☐ The undersigned is an attorney or agent of record. Reg. No. _____	
	
_____ Signature	_____ Date
_____ John Fry Typed or printed name	
_____ General Counsel and Corporate Secretary Title	_____ Telephone Number
☒ Terminal disclaimer fee under 37 CFR 1.20(d) is included.	
WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.	

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875			Application or Docket Number 16/522,237		Filing Date 07/25/2019		☐ To be Mailed	
ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO								
APPLICATION AS FILED - PART I								
		(Column 1)	(Column 2)					
FOR		NUMBER FILED	NUMBER EXTRA		RATE (\$)	FEE (\$)		
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))		N/A	N/A		N/A			
☐ SEARCH FEE (37 CFR 1.16(k), (l), or (m))		N/A	N/A		N/A			
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))		N/A	N/A		N/A			
TOTAL CLAIMS (37 CFR 1.18(j))		minus 20 = *			x \$100 =			
INDEPENDENT CLAIMS (37 CFR 1.18(h))		minus 3 = *			x \$460 =			
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))		If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))								
* If the difference in column 1 is less than zero, enter "0" in column 2.					TOTAL			
APPLICATION AS AMENDED - PART II								
		(Column 1)	(Column 2)		(Column 3)			
AMENDMENT	07/16/2021	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR		PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)	
	Total (37 CFR 1.16(g))	* 21	Minus	** 20	= 1	x \$100 =	100	
	Independent (37 CFR 1.16(n))	* 1	Minus	*** 3	= 0	x \$480 =	0	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
						TOTAL ADD'L FEE		100
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR		PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)	
	Total (37 CFR 1.16(g))	*	Minus	**	=	x \$0 =		
	Independent (37 CFR 1.16(n))	*	Minus	***	=	x \$0 =		
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
						TOTAL ADD'L FEE		
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.						LIE		
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".						/CHRISTINE V MOORE/		
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".								
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.								

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

FORM PTO-1449/A and B (modified PTO/SB/08) INFORMATION DISCLOSURE STATEMENT BY APPLICANT				APPLICATION NO.: 16/522,237		ATTY. DOCKET NO.: Q0052.70018US03	
				FILING DATE: July 25, 2019		CONFIRMATION NO.: 4736	
				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: A. M. Weidner	
Sheet	1	of	1				

U.S. PATENT DOCUMENTS

Examiner's Initials *	Cite No.	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication or Issue of Cited Document MM-DD-YYYY
		Number	Kind Code		
		10,989,713	B2	Rissin et al.	04-27-2021
		2020-0123592	A1	Diaz-Mochon et al.	04-23-2020

FOREIGN PATENT DOCUMENTS

Examiner's Initials *	Cite No.	Foreign Patent Document			Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Translation (Y/N)
		Office/Country	Number	Kind Code			

OTHER ART – NON PATENT LITERATURE DOCUMENTS

Examiner's Initials *	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	Translation (Y/N)
		CAIRNS, A new market beckons for Alzheimer's blood tests. Evaluate Vantage. 2021 Apr 19. https://www.evaluate.com/vantage/articles/analysis/spotlight/new-market-beckons-alzheimers-blood-tests [last accessed 15 July 2021]. 3 pages.	
		DING et al., Ultrasensitive assays for detection of plasma tau and phosphorylated tau 181 in Alzheimer's disease: a systematic review and meta-analysis. Transl Neurodegener. 2021 Mar 12;10(1):10.	
		LILIANG et al., Tau proteins in serum predict outcome after severe traumatic brain injury. J Surg Res. 2010 May 15;160(2):302-7.	
		OST et al., Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury. Neurology. 2006 Nov 14;67(9):1600-4.	
		TEUNISSEN et al., Plasma p-tau217: from 'new kid' to most promising candidate for Alzheimer's disease blood test. Brain. 2020 Dec 5;143(11):3170-3172.	

[NOTE – No copies of U.S. patents, published U.S. patent applications, or pending, unpublished patent applications stored in the USPTO's Image File Wrapper (IFW) system, are included. See 37 CFR § 1.98 and 1287OG163. Copies of all other patent(s), publication(s), unpublished, pending U.S. patent applications, or other information listed are provided as required by 37 CFR § 1.98 unless 1) such copies were provided in an IDS in an earlier application that complies with 37 CFR § 1.98, and 2) the earlier application is relied upon for an earlier filing date under 35 U.S.C. § 120.]

EXAMINER:	DATE CONSIDERED:
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* EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

9410382.1

Electronic Patent Application Fee Transmittal				
Application Number:		16522237		
Filing Date:		25-Jul-2019		
Title of Invention:		METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY		
First Named Inventor/Applicant Name:		David Wilson		
Filer:		Michael Pomianek/Eileen MacKenzie		
Attorney Docket Number:		Q0052.70018US03		
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
SUBMISSION- INFORMATION DISCLOSURE STMT	1806	1	260	260
Total in USD (\$)				260

Electronic Acknowledgement Receipt	
EFS ID:	43273917
Application Number:	16522237
International Application Number:	
Confirmation Number:	4736
Title of Invention:	METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY
First Named Inventor/Applicant Name:	David Wilson
Customer Number:	23628
Filer:	Michael Pomianek/Eileen MacKenzie
Filer Authorized By:	Michael Pomianek
Attorney Docket Number:	Q0052.70018U503
Receipt Date:	16-JUL-2021
Filing Date:	25-JUL-2019
Time Stamp:	20:14:42
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	\$ 260
RAM confirmation Number	E20217FK15051088
Deposit Account	232825
Authorized User	Eileen MacKenzie
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.17 (Patent application and reexamination processing fees)	

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	CAIRNS_Evaluate_Vantage_2021_3pages.pdf	166618	no	3
			9J8F9429D59A0986e4c2d4c0f18197ac39f62b275c		
Warnings:					
Information:					
2	Non Patent Literature	DING_et_al_Transl_Neurodegener_2021_v10_p10.pdf	3891417	no	14
			72a742d01f8baa011e2515c5a02a4550640c04f3		
Warnings:					
Information:					
3	Non Patent Literature	LILIANG_et_al_J_Surg_Res_2010_v160_p302.pdf	281763	no	6
			15a3f0e0b3a711eebc5076ab9990de3ec090ef339		
Warnings:					
Information:					
4	Non Patent Literature	OST_et_al_Neurology_2006_v67_p1600.pdf	312595	no	6
			1967af1fce98420dd1e9ee6754bd8800404118859		
Warnings:					
Information:					
5	Non Patent Literature	TEUNISSEN_et_al_Brain_2020_v143_p3170.pdf	390211	no	3
			11a40661666d092d1bc50969da5701cd0c5f21d551		
Warnings:					
Information:					
6	Miscellaneous Incoming Letter	Q005270018US03-TRN-MJP.pdf	26468	no	1
			1f964620c65509b5bca55b4c51c570ed0a9fb25		
Warnings:					
Information:					

7	Fee Worksheet (SB06)	Q005270018US03-FEE-MJP.pdf	37393 97751c324689955e1725ad7ae5096cd30c777406	no	1
Warnings:					
Information:					
8	Letter specifying the conditions for filing under 37CFR1.97	Q005270018US03-IDSBODY-MJP.pdf	31536 6ed09a80fca5630f5d1f1d54a31dc5eaf67d3640	no	3
Warnings:					
Information:					
9	Information Disclosure Statement (IDS) Form (SB08)	Q005270018US03-IDS1449-MJP.pdf	23929 1f019713e22cc991628f8083187300nab0f1e8a0e	no	1
Warnings:					
Information:					
This is not an USPTO supplied IDS fillable form					
10	Fee Worksheet (SB06)	fee-info.pdf	31131 6d2011aa0795ef5a05096311a0f1b0a2ee9c71548e9	no	2
Warnings:					
Information:					
Total Files Size (in bytes):			5193061		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					

DOCKET NO.: Q0052.70018US03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: David Wilson
Application No.: 16/522,237
Confirmation No.: 4736
Filed: July 25, 2019
For: METHODS OF DETERMINING A TREATMENT PROTOCOL FOR
AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A
BRAIN INJURY
Examiner: A. M. Weidner
Art Unit: 1649

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.8(a)(4).

Dated: July 16, 2021

Electronic Signature for: /Eileen M. MacKenzie/

MAIL STOP AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**STATEMENT FILED PURSUANT TO THE DUTY OF
DISCLOSURE UNDER 37 C.F.R. §§ 1.56, 1.97 AND 1.98**

Sir:

Pursuant to the duty of disclosure under 37 C.F.R. §§ 1.56, 1.97 and 1.98, the undersigned requests consideration of this Information Disclosure Statement.

PART I: Compliance with 37 C.F.R. § 1.97

This Information Disclosure Statement has been filed more than three months after the filing date of this application and after the mailing date of a first Office Action, but before the mailing date of any of a Final Action under 37 C.F.R. § 1.113, a Notice of Allowance under 37 C.F.R. § 1.311, or an action that otherwise closes prosecution in this application.

Please charge our Credit Card in the amount of \$260.00 covering the fee set forth in 37 C.F.R. § 1.17(p).

9410383.1

PART II: Information Cited

The undersigned hereby makes of record in the above-identified application the information listed on the attached form PTO-1449 (modified PTO/SB/08). The order of presentation of the references should not be construed as an indication of the importance of the references.

The undersigned hereby makes the following additional information of record in the above-identified application.

The undersigned would like to bring to the Examiner's attention the following co-pending applications that may contain subject matter related to this application:

<u>Serial No.</u>	<u>Filing Date</u>	<u>Inventor(s)</u>	<u>Docket No.</u>
*17/210,258	03-23-2021	Rissin et al.	Q0052.70015US03

*A copy of this reference is not provided as the Office has waived the requirement under 37 C.F.R. § 1.98(a)(2)(iii) for submitting a copy of a cited U.S. patent application if it is scanned to the Image File Wrapper system and is available on Private PAIR.

PART III: Remarks

Documents cited anywhere in the Information Disclosure Statement are enclosed unless otherwise indicated. It is respectfully requested that:

1. The Examiner consider completely the cited information, along with any other information, in reaching a determination concerning the patentability of the present claims;
2. The enclosed form PTO-1449 (modified PTO/SB/08) be signed by the Examiner to evidence that the cited information has been fully considered by the United States Patent and Trademark Office during the examination of this application;
3. The citations for the information be printed on any patent which issues from this application.

By submitting this Information Disclosure Statement, the undersigned makes no representation that a search has been performed, of the extent of any search performed, or that more relevant information does not exist.

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, in fact, prior art as defined by 35 U.S.C. § 102, or an admission by Applicant as prior art.

Notwithstanding any statements by the undersigned, the Examiner is urged to form his or her own conclusion regarding the relevance of the cited information.

An early and favorable action is hereby requested.

The Director is hereby authorized to charge any deficiency or credit any overpayment in the fees occasioned by the filing of this Information Disclosure Statement to our Deposit Account No. 23/2825 under Docket No. Q0052.70018US03 from which the undersigned is authorized to draw.

Respectfully submitted,

Electronic signature: /Michael J. Pomianek/
Michael J. Pomianek
Registration No.: 46,190
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Docket No.: Q0052.70018US03
Date: July 16, 2021

TRANSMITTAL FORM <i>(to be used for all correspondence after initial filing)</i>	Application Number	16/522,237-Conf. #4736
	Filing Date	July 25, 2019
	First Named Inventor	David Wilson
	Art Unit	1649
	Examiner Name	A. M. Weidner
Total Number of Pages in This Submission	Attorney Docket Number	Q0052.70018US03

ENCLOSURES (Check all that apply)		
☒ Fee Transmittal Form ☐ Fee Attached ☐ Amendment/Reply ☐ After Final ☐ Affidavits/declaration(s) ☐ Extension of Time Request ☐ Express Abandonment Request ☒ Information Disclosure Statement ☐ Certified Copy of Priority Document(s) ☐ Reply to Missing Parts/ Incomplete Application ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53	☐ Drawing(s) ☐ Licensing-related Papers ☐ Petition ☐ Petition to Convert to a Provisional Application ☐ Power of Attorney, Revocation Change of Correspondence Address ☐ Terminal Disclaimer ☐ Request for Refund ☐ CD, Number of CD(s) _____ ☐ Landscape Table on CD	☐ After Allowance Communication to TC ☐ Appeal Communication to Board of Appeals and Interferences ☐ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) ☐ Proprietary Information ☐ Status Letter ☒ Other Enclosure(s) (please Identify below): Form PTO-1449 Copies of cited references
Remarks		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT			
Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Michael J. Pomianek/		
Printed name	Michael J. Pomianek		
Date	July 16, 2021	Reg. No.	46,190

Certificate of Electronic Filing under 37 C.F.R. § 1.8	
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).	
Dated: July 16, 2021	Electronic Signature for: /Eileen M. MacKenzie/

FEE TRANSMITTAL		Complete if known	
☐ Applicant asserts small entity status. See 37 CFR 1.27.		Application Number	16/522,237-Conf. #4736
☐ Applicant certifies micro entity status. See 37 CFR 1.29. Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.		Filing Date	July 25, 2019
		First Named Inventor	David Wilson
		Examiner Name	A. M. Weidner
		Art Unit	1649
TOTAL AMOUNT OF PAYMENT		(\$)	260.00
		Practitioner Docket No.	Q0052.70018US03

METHOD OF PAYMENT (check all that apply)

☐ Check
 ☒ Credit Card
 ☐ Money Order
 ☐ None
 ☐ Other (please identify): _____

☒ Deposit Account
 Deposit Account Number: 23/2825
 Deposit Account Name: Wolf, Greenfield & Sacks, P.C.

For the above-identified deposit account, the Director is hereby authorized to (check all that apply):

☐ Charge fee(s) indicated below
 ☐ Charge fee(s) indicated below, **except for the filing fee**

☒ Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17, **except for any excess claims fees or multiple dependent claim fee**
 ☐ Charge any additional fee(s) or underpayment of fee(s) under 37 CFR 1.16 and 1.17

☒ Credit any overpayment of fee(s)

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES (U = undiscounted fee; S = small entity fee; M = micro entity fee)

Application Type	FILING FEES			SEARCH FEES			EXAMINATION FEES			Fees Paid (\$)
	U (\$)	S (\$)	M (\$)	U (\$)	S (\$)	M (\$)	U (\$)	S (\$)	M (\$)	
Utility	320	160*	80	700	350	175	800	400	200	
Design	220	110	55	160	80	40	640	320	160	
Plant	220	110	55	440	220	110	660	330	165	
Reissue	320	160	80	700	350	175	2,320	1,160	580	
Provisional	300	150	75	0	0	0	0	0	0	

* The \$160 small entity status filing fee for a utility application is further reduced to \$80 for a small entity status applicant who files the application via EFS-Web.

2. EXCESS CLAIM FEES

Fee Description	Undiscounted Fee (\$)	Small Entity Fee (\$)	Micro Entity Fee (\$)
Each claim over 20 (including Reissues)	100	50	25
Each independent claim over 3 (including Reissues)	480	240	120
Multiple dependent claims	860	430	215

Total Claims **Extra Claims** **Fee (\$)** **Fee Paid (\$)** **Multiple Dependent Claims** **Fee (\$)** **Fee Paid (\$)**

HP = _____ x _____ = _____
 HP = highest number of total claims paid for, if greater than 20.

Indep. Claims **Extra Claims** **Fee (\$)** **Fee Paid (\$)**

HP = _____ x _____ = _____
 HP = highest number of independent claims paid for, if greater than 3.

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is \$420 (\$210 for small entity) (\$105 for micro entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee (\$)	Fee Paid (\$)
_____	_____	_____	_____	_____

_____ - 100 = _____ / 50 = _____ (round up to a whole number) x _____ = _____

4. OTHER FEE(S)

	Fees Paid (\$)
Non-English specification, \$130 fee (no small or micro entity discount)	
Non-electronic filing fee under 37 CFR 1.16(t) for a utility application, \$400 fee (\$200 small or micro entity)	
Other (e.g., late filing surcharge): 1806 Submission of an Information Disclosure Statement	260.00

SUBMITTED BY

Signature	/Michael J. Pomianek/	Registration No. (Attorney/Agent)	46.190	Telephone	617.646.8000
Name (Print/Type)	Michael J. Pomianek	Date	July 16, 2021		

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: July 16, 2021 Electronic Signature for: /Eileen M. MacKenzie/

Application Number * 16/522,237 *	Application/Control No. 16/522,237	Applicant(s)/Patent under Reexamination Wilson et al.	
	Examiner WEIDNER, ADAM M	Art Unit 1649	
Document Code - DISQ		Internal Document - DO NOT MAIL	

TERMINAL DISCLAIMER	☒ APPROVED	☐ DISAPPROVED
Date Filed: <u>16 July 2021</u>	This patent is subject to a Terminal Disclaimer	

Approved/Disapproved by:
/PAMELA A YOUNG/ Technology Center: <u>OPLC</u> Telephone: <u>(571)272-3622</u> <u>2 TD</u>



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/522,237	07/25/2019	David Wilson	Q0052.70018US03	4736
23628	7590	08/02/2021	EXAMINER	
WOLF GREENFIELD & SACKS, P.C. 600 ATLANTIC AVENUE BOSTON, MA 02210-2206			WEIDNER, ADAM M	
			ART UNIT	PAPER NUMBER
			1649	
			NOTIFICATION DATE	DELIVERY MODE
			08/02/2021	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

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<i>Applicant-Initiated Interview Summary</i>	Application No. 16/522,237	Applicant(s) Wilson et al.		
	Examiner ADAM M WEIDNER	Art Unit 1649	AIA (First Inventor to File) Status No	Page 1 of 2

All Participants (applicant, applicants representative, PTO personnel)	Title	Type
ADAM M WEIDNER	Primary Examiner	Telephonic
Michael Pomianek	Attorney of Record	
Andrew Maher	Attorney	
David Duffy	CTO of Applicant	

Date of Interview: 30 June 2021

Issues Discussed:

35 U.S.C. 103

Discussed obviousness for using the assay in a specific clinical application versus whether or not the assay was reasonably expected to be able to detect tau.

Discussed secondary considerations of commercial success. Discussed the Ding reference, noting many groups used the claimed assay to detect tau but only after the instant effective filing date.

Representative briefly indicated the Quaternix document might not be prior art, but did not pursue this further.

Representatives indicated that the Quaternix document could not have been actually detecting tau, as 50 pg/ml is too much.

Discussed proposed claim 105, indicating this was an improvement over the prior art implementation of SiMOa.

Examiner agreed to consider the evidence of secondary considerations. Further, the Examiner agreed that if claim 105 was a non-obvious improvement as alleged, then the claim might be sufficient to overcome the 103 rejection.

☒ Attachment

/Adam Weidner/ Primary Examiner, Art Unit 1649	
Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04 Please further see: MPEP 713.04 Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b) 37 CFR § 1.2 Business to be transacted in writing	

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview.

<i>Applicant-Initiated Interview Summary</i>	Application No. 16/522,237	Applicant(s) Wilson et al.		
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Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

Docket No.: Q0052.70018US03
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: David Wilson
Application No.: 16/522,237
Confirmation No.: 4736
Filed: July 25, 2019
For: METHODS OF DETERMINING A TREATMENT PROTOCOL FOR
AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A
BRAIN INJURY
Examiner: A. M. Weidner
Art Unit: 1649

PROPOSED INTERVIEW AGENDA

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Examiner Weidner:

Thank you for agreeing to discuss the above-referenced application with us via telephone conference to be held on Wednesday, June 30, 2021 at 2:30 PM (EDT).

The undersigned will be joined by Representative Andrew G. Maher (Applicant's representative; Reg. No. 79,399) and Dr. David C. Duffy (Chief Technology Officer of the Applicant, Quanterix Corporation). Dr. Duffy is authorized to make binding statements on behalf the Applicant.

During the interview, we plan to discuss the rejection of claim 92 under 35 U.S.C. 103 over US20100075407 ("Duffy '407") in view of Quanterix 2010 IQT Technology Focus Day ("Quanterix 2010a"). Dr. Duffy is the first named inventor of Duffy '407 and was the presenter of the Quanterix 2010a slides. As part of the discussion, we would like to discuss potential amendments to the claims, attached as Appendix A. The claims in Appendix A include new dependent claim 105, which recites further details of the analytical protein concentration measurement assay used in step (C) and which further distinguishes the cited references of record

We anticipate discussing the success and recognized importance of the claimed method, especially in view of the growing market for blood-based analytical tau assays for patients suspected of having neurological conditions. In this context we plan on referring to a recent meta-analysis review article on ultrasensitive plasma tau assays by Ding et al. (Ding, et al. (2021). "Ultrasensitive assays for detection of plasma tau and phosphorylated tau 181 in Alzheimer's disease: a systematic review and meta-analysis." *Translational neurodegeneration*, 10(1), 1-14; attached as Appendix B) and a recent commentary on the promise of plasma tau assays for Alzheimer's disease blood tests by Teunissen et al. (Teunissen, et al. (2020). "Plasma p-tau217: from 'new kid' to most promising candidate for Alzheimer's disease blood test." *Brain*, 143(11), 3170-3172; attached as Appendix C).

Additionally, we plan on discussing the nature, circumstances, and content of the Quanterix 2010a reference, including slide 22. We expect the discussion will also include what a person of ordinary skill in the art at the time would have understood and reasonably expected regarding analytical quantification of tau in blood based on Quanterix 2010a and other references cited in the record.

We look forward to our interview.

Dated: June 29, 2021

Respectfully submitted,

By: /Michael J. Pomianek/
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APPENDIX A: Claim Amendments for Discussion During Interview

1. - 10. (Canceled)

11. (Previously Presented) The method of claim 92, wherein the brain injury results from a hypoxic event.

12. - 19. (Canceled)

20. (Previously Presented) The method claim 92, wherein the limit of detection for the analytical protein concentration measurement assay is less than about 0.02 pg/mL.

21. (Previously Presented) The method of claim 92, wherein the brain injury comprises a hypoxic event caused by cardiac arrest.

22. (Previously Presented) The method of claim 92, wherein the brain injury comprises a hypoxic event caused by stroke.

23. (Previously Presented) The method of claim 92, wherein the brain injury comprises a hypoxic event caused by an ischemic event.

24. (Previously Presented) The method of claim 92, wherein the brain injury comprises a hypoxic event caused by a thrombosis.

25. - 91. (Cancelled)

92. (Currently Amended) A method of producing a body fluid sample of a patient ~~recovering from a brain injury~~ suspected of having a neurological condition containing an analytically quantified amount of endogenous tau protein, said method comprising:

(A) obtaining a volume of body fluid comprising blood, or a blood component selected from plasma and serum,

(B) diluting the volume of body fluid, and

(C) quantifying through the use of an analytical protein concentration measurement assay a concentration of tau protein in the volume of body fluid to produce the body fluid sample containing the analytically quantified amount of endogenous tau protein;

wherein the analytically quantified concentration of endogenous tau protein contained in the body fluid sample is less than about 5 pg/ml, and a limit of quantification of the analytical protein concentration measurement assay used in step (C) for quantifying the concentration of tau protein is less than about 0.2 pg/mL.

93. (Previously Presented) The method of claim 92, wherein the analytical protein concentration measurement assay used in step (C) comprises:

i. exposing a plurality of capture objects that each include a binding surface having affinity for tau protein molecules to the volume of body fluid;

ii. immobilizing at least a portion of the tau protein molecules with respect to the plurality of capture objects such that at least some of the capture objects associate with at least one tau protein molecule and a statistically significant fraction of the capture objects do not associate with any tau protein molecules;

iii. determining a proportion of the plurality of capture objects that associated with a tau protein molecule in step ii; and

iv. determining a measure of tau protein molecule concentration in the volume of body fluid based at least in part on the proportion determined in step iii.

94. (Previously Presented) The method of claim 93, wherein the plurality of capture objects comprises a plurality of beads.

95. (Previously Presented) The method of claim 92, wherein the analytical protein concentration measurement assay used in step (C) comprises spatially segregated tau protein molecules into a plurality of locations, wherein a statistically significant fraction of the locations

contain a single tau protein molecule and a statistically significant fraction of the locations do not contain any tau protein molecules

96. (Previously Presented) The method of claim 95, wherein the plurality of locations comprises a plurality of reaction vessels.

97. (Previously Presented) The method of claim 92, wherein each of the tau protein molecules quantified in step (C) is selected from the group consisting of tau 23 (352, 0N3R), tau 24 (383, 0N4R), tau 37 (381, 1N3R), tau 34 (412, 1N4R), tau 39 (410, 2N3R), and tau 40 (441, 2N4R).

98. (Previously Presented) The method of claim 97, wherein at least a portion of the tau protein molecules are phosphorylated.

99. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid comprises blood.

100. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid comprises plasma.

101. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid comprises serum.

102. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid does not comprise cerebrospinal fluid (CSF).

103. (Currently Amended) The method of claim 104 [[92]], wherein the has been collected from the patient within 12 hours of the brain injury.

104. (New) The method of claim 92, wherein the patient suspected of having a neurological condition is recovering from a brain injury.

105. (New) The method of claim 92, wherein the analytical protein concentration measurement assay used in step (C) comprises:

- i. suspending a plurality of capture objects that each include a binding surface having affinity for tau protein molecules in the diluted volume of body fluid,
- ii. immobilizing at least a portion of the tau protein molecules with respect to at least some of the plurality of capture objects suspended in the diluted volume of body fluid such that at least some of the capture objects associate with at least one tau protein molecule from the volume of the body fluid, and
- iii. interrogating at least some of the capture objects associated with at least one tau molecule from the volume of the body of fluid; and
- iv. determining a measure of tau protein molecule concentration in the volume of body fluid based at least in part on the interrogating step performed in step iii.

APPENDIX B: Ding et al., *Translational Neurodegeneration* (2021)

REVIEW

Open Access



Ultrasensitive assays for detection of plasma tau and phosphorylated tau 181 in Alzheimer's disease: a systematic review and meta-analysis

Xulong Ding^{1†}, Shuting Zhang^{2†}, Lijun Jiang³, Lu Wang⁴, Tao Li^{3*} and Peng Lei^{3*}✉

Abstract

A lack of convenient and reliable biomarkers for diagnosis and prognosis is a common challenge for neurodegenerative diseases such as Alzheimer's disease (AD). Recent advancement in ultrasensitive protein assays has allowed the quantification of tau and phosphorylated tau proteins in peripheral plasma. Here we identified 66 eligible studies reporting quantification of plasma tau and phosphorylated tau 181 (ptau181) using four ultrasensitive methods. Meta-analysis of these studies confirmed that the AD patients had significantly higher plasma tau and ptau181 levels compared with controls, and that the plasma tau and ptau181 could predict AD with high-accuracy area under curve of the Receiver Operating Characteristic. Therefore, plasma tau and plasma ptau181 can be considered as biomarkers for AD diagnosis.

Keywords: Tau, Phosphorylated tau 181, Alzheimer's disease, Simoa, IMR, EIMAF/a-EIMAF, MSD, Plasma biomarker

Background

There is no cure for Alzheimer's disease (AD). The lack of early diagnostic biomarkers for selecting prodromal or early-stage patients is one of the roadblocks in clinical trials. The National Institute on Aging—Alzheimer's Association (NIA-AA) has recently proposed a research framework for AD and specified the importance of amyloid-beta (A β), tau, and neurodegeneration [AT(N)] in the biological definition of AD [1]. Although the A β - or tau-positron emission tomography (PET) has been developed, it is yet to be globally available, making the

NIA-AA research framework challenging to put into practice. Therefore, it is urgent to discover convenient biomarkers with early-diagnostic significance.

Tau is a microtubule-associated protein localized primarily in neurons. It is also a primary component of neurofibrillary tangles (NFTs), a pathological hallmark in AD [2]. The loss of normal functions and the gain of toxic functions of Tau have been linked with the pathogenesis of AD [2–7]. Mounting evidence has suggested that the cerebrospinal fluid (CSF) levels of tau and phosphorylated tau are linearly associated with symptom severity of AD [8–11], suggesting tau as a promising biomarker for early diagnosis and prognostic prediction. However, clinical application of CSF biomarkers has been hindered by high cost, invasiveness, and side effects of lumbar punctures, such as positional headache [12].

The detection of tau in plasma has been limited due to its low abundance until recent technical development of ultrasensitive assays. The plasma tau or phosphorylated

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tau levels in the healthy population and diseased patients have been assessed using different technologies such as Single-molecule Array (Simoa) [13], ImmunoMagnetic Reduction (IMR) [14], enhanced immunoassay using multi-arrayed fiber optics conjugated with rolling circle amplification (a-EIMAF) [15] and Meso Scale Discovery (MSD) [16]. However, the plasma tau or phosphorylated tau levels vary among studies, and there is no cut-off threshold between AD and normal elderly. In this systematic review and meta-analysis, we set out to determine the normal range of plasma tau and phosphorylated tau 181 (ptau181) levels in healthy populations stratified by age and sex, and investigate the cut-off thresholds of plasma tau and ptau181 between AD patients and controls.

Methods

Literature search

Literature search was performed in databases Cochrane Library, MEDLINE, EMBASE, PubMed, Web of Science, and Google Scholar by the date of January 21st, 2021, according to the methodology suggested by the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines [17], using the following terms: Alzheimer disease, AD, dementia, Parkinson disease, PD, traumatic brain injury, TBI, plasma tau, plasma total tau, plasma phosphorylated tau, Simoa IMR, EIMAF, a-EIMAF, and MSD. Papers published in an online-first and ahead-of-print manner were included in the analysis. The protocol of the overarching project has been published (PROSPERO registration No. CRD42020151852).

Study selection

The analysis involved three questions. For Question 1, i.e., the normal range of plasma tau/ptau181 in healthy populations, the following criteria were applied: (1) full-text publications in English; and (2) plasma tau and/or ptau181 levels were measured by Simoa, IMR, EIMAF/a-EIMAF, or MSD. Studies were excluded if: (1) without sufficient data to allow for the extraction of plasma tau/ptau181 levels, or (2) the mean age of cohorts in the studies was < 18 years.

For Question 2, i.e., the differences of plasma tau/ptau181 between AD patients and controls, the following selection criteria were applied: (1) full-text publications in English; (2) plasma tau and/or ptau181 levels were measured by Simoa, IMR, EIMAF/a-EIMAF, or MSD; and (3) AD was diagnosed according to the 2011 core clinical NIA-AA [18] or the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association [19] guidelines. Studies were excluded if: (1) studies without sufficient data to allow for the extraction of

plasma tau/ptau181 levels, (2) the mean age of cohorts in the studies was < 18 years, or (3) patients employed in the studies had other cognitive disorders (e.g., mild cognitive impairment [MCI], vascular dementia, and frontotemporal dementia).

Question 3 is to calculate the diagnostic accuracy of plasma tau/ptau181 for AD. For this, we analyzed all publications selected for Question 2, if the reported plasma tau/ptau181 were used for AD diagnosis in the original publications.

Data extraction and quality assessment

Two authors (XLD and STZ) performed the study assessment independently, and data were reported following the PRISMA statement [17]. Any discrepancy would be discussed with additional reviewers (LJ and LW). Study quality was assessed using the modified version of the Newcastle-Ottawa Scale (NOS) [20]. A score of up to 8 points was assigned to each study based on the quality of population selection, the comparability between groups, and assessment of exposure. For studies with diagnostic tests, we assessed the quality of selected literature by checking items of the Quality Assessment of Diagnostic Accuracy Studies-2 tool (QUADAS-2) [21].

Statistical analysis and heterogeneity exploration

For the normal range of plasma tau or ptau181, the effect sizes and 95% confidence intervals (CI) were calculated using the Random-effect model, which was used to presume that the true effect size varied among studies [21]. Subgroup analysis was performed according to age and sex ratio. For the diagnostic value of plasma tau or ptau181, the weighted mean difference (WMD) with 95% CI was calculated between AD patients and controls with a random-effects model. To evaluate the diagnostic test accuracy, we used the diagnostic accuracy studies module [22] to calculate main outcome measures, including sensitivity and specificity, and the diagnostic odds ratio (DOR). We then fitted a hierarchical summary receiver operating characteristic (HSROC) model using a bivariate regression approach to visualize the relationship between sensitivity and specificity and calculated the area under curve (AUC). Heterogeneity among studies was assessed using the I^2 index. According to the Cochrane handbook, the heterogeneity was classified as low (I^2 index 0%–40%), moderate (30%–60%), substantial (50%–90%), and considerable (75%–100%) [23, 24]. For heterogeneity exploration, meta-regression analysis was performed to evaluate the potential bias factors, while age, sample size, sex ratio, and region were included as four covariates. Furthermore, the results of meta-analyses were assessed by funnel plots and Egger's test [25]. All calculations were carried out using statistical softwares provided by the Cochrane Collaboration (RevMan 5.1) and Stata14.1

(StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

Results

Study inclusions and quality assessment

The search strategy identified 4230 studies without duplication, and 2999 studies were excluded after abstract screening. Of the left 85 studies with full-text content, 19 studies were excluded as they were not a clinical study or lacked a clinical outcome description. Finally 66 studies were included in this meta-analysis (Fig. 1). Based on the modified NOS criteria [20], 53 studies were classified as high-quality, and 13 studies as medium-quality (Table S1). The revised QUADAS-2 [26] determined that the overall quality of studies included was robust. Most studies ranked as low bias and low applicability concern (Fig. S1).

The characteristics, including number of subjects, average age, male percentage, and plasma tau/ptau181

levels of the 66 studies are summarized in Tables 1 and 2. Forty-one studies using Simoa (15,490 healthy controls), 15 studies using IMR (727 healthy controls), and two studies using a-EIMAF (189 healthy controls) were included for plasma tau studies. Twenty-two studies focused on AD, comprising 1456 patients with AD and 1973 controls. Only studies using Simoa (13 studies, 1189 AD patients and 1611 controls) and IMR (9 studies, 267 AD patients and 362 controls) were included for analysis since there were insufficient data for a-EIMAF. For ptau181, six studies using the Simoa - Karikari method (not the commercial p-tau181 version) (1424 healthy controls) and three using MSD (440 healthy controls) were included for the normal range analysis. Four studies using Simoa (392 AD patients and 773 controls) and three studies using MSD (231 AD patients and 440 controls) were identified for analysis related to AD diagnosis.

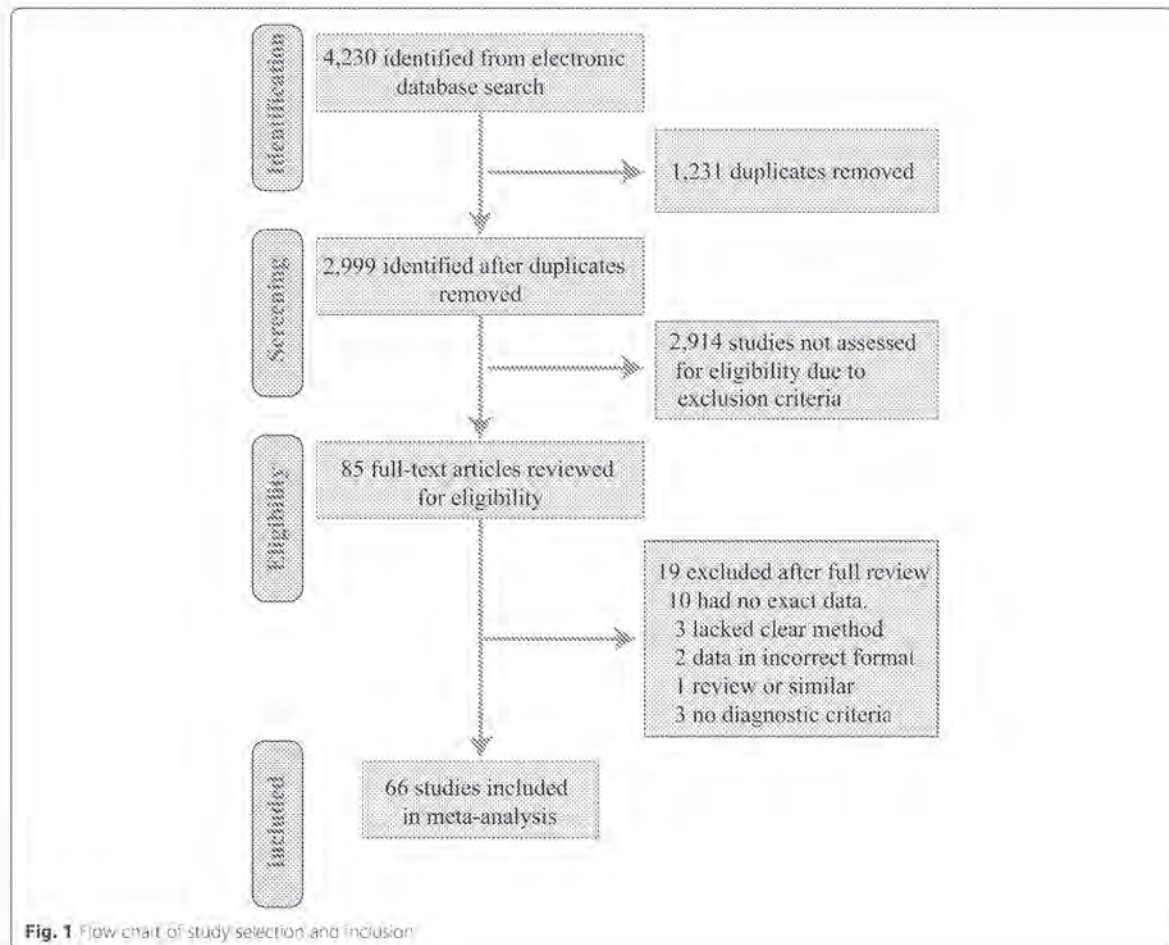


Table 1 Basic characteristics of included studies for plasma tau analysis

Study	Subjects (n)	Male (%)	Age, years (mean $\pm$ SD)	Plasma tau (pg/ml)	Research question	Method
Shahim et al. (2014) [27]	CN (47)	NA	28 $\pm$ 14.07	4.5 $\pm$ 5.66	1	Simoa
Bogoslovsky et al. (2015) [28]	CN (69)	51	45 $\pm$ 15.5	4.34 $\pm$ 1.77	1	Simoa
Olivera et al. (2015) [29]	CN (28)	96.4	28.40 $\pm$ 4.47	0.63 $\pm$ 0.48	1	Simoa
Dage et al. (2016) [30]	CN (378)	61.4	80 $\pm$ 5.19	4.14 $\pm$ 1.56	1	Simoa
Alosco et al. (2017) [31]	CN (25)	100	55.16 $\pm$ 7.95	2.46 $\pm$ 0.57	1	Simoa
Mielke et al. (2017) [32]	CN (335)	62.4	80.8 $\pm$ 4.8	4.2 $\pm$ 1.5	1	Simoa
Müller et al. (2017) [33]	CN (134)	59.7	68.4 $\pm$ 5	3.6 $\pm$ 1.7	1	Simoa
Kasai et al. (2017) [34]	CN (22)	54.5	37.4 $\pm$ 12.0	0.470 $\pm$ 0.232	1	Simoa
Foiani et al. (2018) [35]	CN (22)	41	68.7 $\pm$ 6.5	1.67 $\pm$ 0.50	1	Simoa
Bergman et al. (2018) [36]	CN (36)	0	30 $\pm$ 4	6.24 $\pm$ 2.76	1	Simoa
Lippa et al. (2018) [37]	CN (42)	90.5	36.21 $\pm$ 11.69	2.81 $\pm$ 1.20	1	Simoa
Verberk et al. (2018) [38]	CN (191)	63	59 $\pm$ 9	3.18 $\pm$ 1.07	1	Simoa
Shahim et al. (2018) [39]	CN (19)	NA	25.0 $\pm$ 8.89	1.8 $\pm$ 1.48	1	Simoa
Wallace et al. (2018) [40]	CN (13)	100	18.5 $\pm$ 1.7	2.56 $\pm$ 1.02	1	Simoa
Motamedi et al. (2018) [41]	CN (24)	91.7	30.9 $\pm$ 7.77	2.48 $\pm$ 1.94	1	Simoa
Fortea et al. (2018) [42]	CN (67)	30	52.05 $\pm$ 5.50	2.23 $\pm$ 1.63	1	Simoa
Zeitberger et al. (2018) [43]	CN (13)	46.2	37	2.08 $\pm$ 1.23	1	Simoa
Shi et al. (2019) [44]	CN (87)	41.4	64.77 $\pm$ 7.40	3.56 $\pm$ 1.84	1	Simoa
Kitaguchi et al. (2019) [45]	CN (11)	45.4	67.8 $\pm$ 3.71	0.63 $\pm$ 0.3	1	Simoa
Pase et al. (2019) [46]	CN (3232)	46.8	58 $\pm$ 14	3.93 $\pm$ 1.11	1	Simoa
Korley et al. (2019) [47]	CN (63)	63.5	39.0 $\pm$ 20.7	3.5 $\pm$ 3.19	1	Simoa
Kritikos et al. (2020) [48]	CN (398)	94.72	54.3 $\pm$ 8.1	1.67 $\pm$ 0.685	1	Simoa
Wolf et al. (2020) [49]	CN (4444)	42	71.9 $\pm$ 7.5	2.6 $\pm$ 2.3	1	Simoa
Verberk et al. (2020) [50]	CN (241)	60	61.9 $\pm$ 10	3.1 $\pm$ 1	1	Simoa
Pattinson et al. (2020) [51]	CN (18)	85.3	35.56 $\pm$ 12.39	2.57 $\pm$ 1.07	1	Simoa
Romero et al. (2020) [52]	CN (3472)	46	54.9 $\pm$ 13.2	3.9 $\pm$ 1.1	1	Simoa
Petersen et al. (2020) [53]	CN (225)	51.5	45.7 $\pm$ 7.1	2.4 $\pm$ 1.8	1	Simoa
Cantero et al. (2020) [54]	CN (57)	47.4	67.7 $\pm$ 3.4	3.1 $\pm$ 1.5	1	Simoa
Zetterberg et al. (2013) [55]	CN (25)	24	74 $\pm$ 6.7	4.43 $\pm$ 2.83	1 and 2	Simoa
	AD (54)	31.5	75 $\pm$ 6.2	8.80 $\pm$ 10.1		
Mattsson et al. (2016) ADNI [56]	CN (189)	55	75.9 $\pm$ 4.9	2.58 $\pm$ 1.19	1 and 2	Simoa
	AD (179)	52	75.2 $\pm$ 7.4	3.12 $\pm$ 1.50		
Mattsson et al. (2016) BioFINDER [56]	CN (274)	39	72.9 $\pm$ 4.9	5.58 $\pm$ 2.51	1 and 2	Simoa
	AD (61)	42	76.4 $\pm$ 4.7	5.37 $\pm$ 2.56		
Shi et al. (2016) [44]	CN (106)	54.7	67.1 $\pm$ 7.4	2.405 $\pm$ 2.76	1, 2 and 3	Simoa
	AD (106)	53.8	69.5 $\pm$ 8.1	3.870 $\pm$ 2.22		
Kovács et al. (2017) [57]	CN (18)	50	73.7	1.68 $\pm$ 0.17	1 and 2	Simoa
	AD (21)	23.8	77	7.5 $\pm$ 3.2		
Deters et al. (2017) [58]	CN (166)	57.2	75.2 $\pm$ 5.1	2.71 $\pm$ 1	1 and 2	Simoa
	AD (168)	51.8	75.3 $\pm$ 7.3	3.13 $\pm$ 1.3		
Chen et al. (2017) [59]	CN (151)	58.9	75.7 $\pm$ 4.9	2.7 $\pm$ 1.1	1 and 2	Simoa
	AD (149)	55.7	76.1 $\pm$ 7.3	3.2 $\pm$ 1.3		
Mielke et al. (2018) [60]	CN (172)	69.2	71.9 $\pm$ 9.5	5.9 $\pm$ 1.90	1 and 2	Simoa

Table 1 Basic characteristics of included studies for plasma tau analysis (Continued)

Study	Subjects (n)	Male (%)	Age, years (mean $\pm$ SD)	Plasma tau (pg/ml)	Research question	Method
Park et al. (2019) [61]	AD (40)	23	67.7 $\pm$ 9.2	7.2 $\pm$ 2.80	1, 2 and 3	Simoa
	CN (172)	40.4	71.08 $\pm$ 1.0	2.37 $\pm$ 0.1		
	AD (40)	13.3	75.87 $\pm$ 2.1	3.36 $\pm$ 0.3		
Li et al. (2019) [62]	CN (9)	44.4	61.78 $\pm$ 10.52	4.62 $\pm$ 0.50	1 and 2	Simoa
	AD (53)	42.3	68.39 $\pm$ 9.65	5.47 $\pm$ 2.69		
Startin et al. (2019) [63]	CN (27)	59.3	49.26 $\pm$ 10.40	1.49 $\pm$ 1.26	1 and 2	Simoa
	AD (27)	66.7	59.33 $\pm$ 4.04	1.45 $\pm$ 1.02		
Sugarman et al. (2020) [64]	CN (238)	37.4	72.38 $\pm$ 7.69	3.22 $\pm$ 2.73	1, 2 and 3	Simoa
	AD (156)	55.8	76.74 $\pm$ 8.12	3.73 $\pm$ 3.07		
Fossati et al. (2020) [65]	CN (68)	35.3	67.71 $\pm$ 8.54	2.74 $\pm$ 0.76	1, 2 and 3	Simoa
	AD (29)	34.5	72.81 $\pm$ 9.69	3.67 $\pm$ 1.06		
Deniz et al. (2020) [66]	CN (162)	23.5	82.7 $\pm$ 8.15	3.84 $\pm$ 2.27	1 and 2	Simoa
	AD (159)	31.2	78.2 $\pm$ 8.97	3.75 $\pm$ 2.36		
Lin et al. (2018) [67]	CN (35)	40.0	62.6 $\pm$ 9.7	12.12 $\pm$ 0.96	1	IMR
Chi et al. (2019) [68]	CN (42)	79	59 $\pm$ 11.1	18.2 $\pm$ 13.78	1	IMR
Chen et al. (2019) [69]	CN (13)	69.2	73 $\pm$ 13.0	18.7 $\pm$ 5.63	1	IMR
Chen et al. (2020) [70]	CN (28)	28.6	61.1 $\pm$ 4.9	14.67 $\pm$ 8.49	1	IMR
Fang et al. (2020) [71]	CN-young (43)	67	38.7 $\pm$ 13.5	14.9 $\pm$ 5.5	1	IMR
	CN-old (34)	41	70.3 $\pm$ 5.8	15.0 $\pm$ 7.3		
Chiu et al. (2017) [72]	CN-middle (56)	29.1	58.1 $\pm$ 4.9	14.35 $\pm$ 6.49	1	IMR
	CN-old (70)	45.7	73.6 $\pm$ 6.3	18.14 $\pm$ 7.33		
Chiu et al. (2013) [73]	CN (30)	43.3	64.4 $\pm$ 9.5	15.6 $\pm$ 6.9	1 and 2	IMR
	AD (10)	40	69.3 $\pm$ 9.4	53.9 $\pm$ 11.7		
Tzen et al. (2014) [74]	CN (20)	50	63.7 $\pm$ 7.9	13.5 $\pm$ 5.5	1 and 2	IMR
	AD (14)	28.6	64.9 $\pm$ 11.5	46.7 $\pm$ 2.0		
Yang et al. (2017) [75]	CN (66)	NA	64.6 $\pm$ 8.6	13.37 $\pm$ 7.77	1 and 2	IMR
	AD (29)		72.2 $\pm$ 9.9	55.44 $\pm$ 22.45		
Lee et al. (2017) [76]	CN-young (44)	68.1	38.4 $\pm$ 13.5	14.9 $\pm$ 5.5	1 and 2	IMR
	CN-old (34)	41.2	70.3 $\pm$ 5.8	15.0 $\pm$ 7.3		
	AD (62)	46.8	72.1 $\pm$ 11.1	47.5 $\pm$ 18.9		
Lue et al. (2017) BSHRI [77]	CN (16)	25	81.9 $\pm$ 1.5	20.48 $\pm$ 1.24	1 and 2	IMR
	AD (16)	43.8	82.5 $\pm$ 1.4	34.52 $\pm$ 3.75		
Lue et al. (2017) NTUH [77]	CN (61)	39.3	64.2 $\pm$ 1.1	13.98 $\pm$ 1.89	1 and 2	IMR
	AD (31)	54.8	72.5 $\pm$ 1.8	52.47 $\pm$ 2.72		
Yang et al. (2018) [78]	CN (23)	NA	67.5 $\pm$ 7.1	18.85 $\pm$ 10.16	1 and 2	IMR
	AD (21)		78.8 $\pm$ 7.9	37.54 $\pm$ 12.29		
Chiu et al. (2019) BSHRI [79]	CN (16)	NA	81.9 $\pm$ 6	20.48 $\pm$ 4.96	1 and 2	IMR
	AD (16)		82.5 $\pm$ 1.4	34.52 $\pm$ 1.4		
Chiu et al. (2019) NTUH [79]	CN (37)	NA	66.1 $\pm$ 8.3	16.61 $\pm$ 9.18	1 and 2	IMR
	AD (25)		78.1 $\pm$ 7.3	43.35 $\pm$ 15.14		
Liao et al. (2020) [80]	CN (57)	45.6	67.9 $\pm$ 9.5	20.65 $\pm$ 3.52	1 and 2	IMR

Table 1 Basic characteristics of included studies for plasma tau analysis (Continued)

Study	Subjects (n)	Male (%)	Age, years (mean $\pm$ SD)	Plasma tau (pg/ml)	Research question	Method
Liu et al. (2020) [81]	AD (40)	37.5	68.1 $\pm$ 9.0	25.91 $\pm$ 8.12	1 and 2	IMR
	CN (2)	50	37 $\pm$ 2.8	17.4 $\pm$ 1.1		
	AD (3)	33.3	71.3 $\pm$ 4.0	28.3 $\pm$ 4.0		
Rubenstein et al. (2017) [82]	CN (20)	70	40.5 $\pm$ 14.2	0.063 $\pm$ 0.014	1	a-EIMAF
Gardner et al. (2018) [83]	CN-young (79)	73.4	25.8 $\pm$ 7.3	0.079 $\pm$ 0.0087	1	a-EIMAF
	CN-middle-aged (60)	66.7	50.0 $\pm$ 5.9	0.078 $\pm$ 0.008		
	CN-older (30)	63.3	68.0 $\pm$ 8.4	0.079 $\pm$ 0.0065		

Plasma tau and ptau181 levels in healthy cohorts

Subgroup analysis was performed according to age and sex ratio, among studies using Simoa, IMR, a-EIMAF, and MSD, respectively. Based on the data retrieved, the average age of cohort stratification was classified as young (< 40 years old), middle-aged (40–60 years old), and old (> 60

years). The sex ratio was defined as low (less than 40% male), middle (40%–60% male), and high (over 60% male).

Among the 66 studies, 58 studies that used Simoa, IMR, or a-EIMAF were included for the analysis of normal range of plasma tau, as the MSD studies focused on CSF tau. Forty-one publications, reporting 42

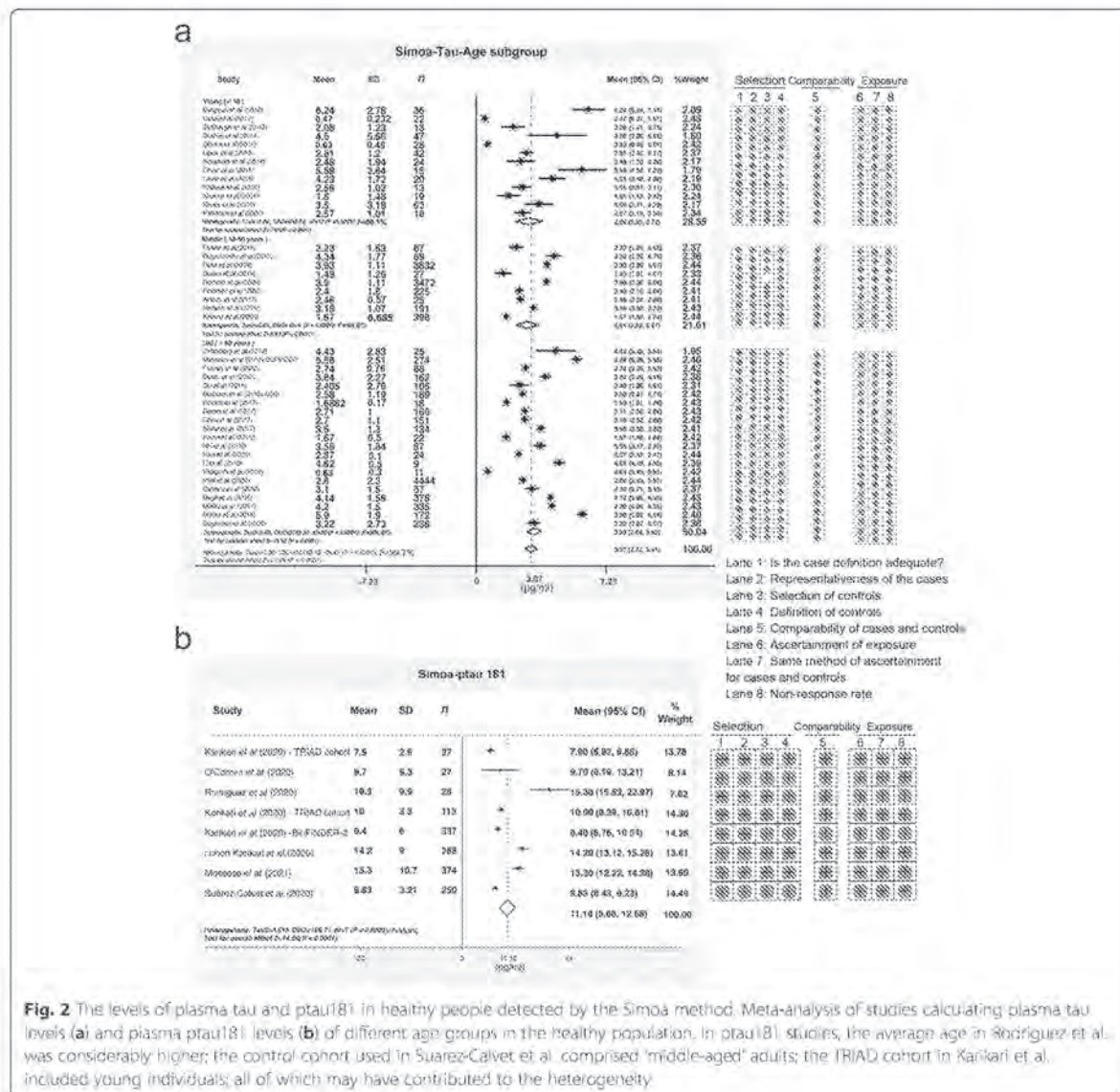
Table 2 Basic characteristics of included studies for plasma ptau181 analysis

Study	Subjects (n)	Male (%)	Age, years (mean $\pm$ SD)	P-tau-181 (pg/ml)	Research question	Method
Suárez-Calvet et al. (2020) [94]	CN (250)	38	60.6 $\pm$ 4.44	8.83 $\pm$ 3.21	1	Simoa - Karikari method
Moscato et al. (2021) [85]	CN (374)	47.1	74.8 $\pm$ 6.6	13.3 $\pm$ 10.7	1	Simoa - Karikari method
O'Connor et al. (2020) [95]	CN (27)	41	38.1 $\pm$ 10.7	9.7 $\pm$ 9.3	1, 2 and 3	Simoa - Karikari method
	AD (19)	63	50.7 $\pm$ 10.0	23.7 $\pm$ 10.5		
Rodríguez et al. (2020) [87]	CN (28)	35.7	82.2 $\pm$ 6.5	19.3 $\pm$ 9.9	1, 2 and 3	Simoa - Karikari method
	AD (77)	46.1	81.7 $\pm$ 7.6	28.4 $\pm$ 9.6		
Karikari et al. (2020) - FRAD cohort [88]	CN-young (27)	37	22.7 $\pm$ 1.9	7.9 $\pm$ 2.6	1, 2 and 3	Simoa - Karikari method
	CN-old (113)	36	69.2 $\pm$ 9.7	10 $\pm$ 3.3		
	AD (33)	55	64.6 $\pm$ 9.2	24.9 $\pm$ 7.8		
Karikari et al. (2020) - BioFINDER-2 cohort [96]	CN-old (337)	46	63.1 $\pm$ 5.0	9.4 $\pm$ 6.0	1, 2 and 3	Simoa - Karikari method
	AD (126)	47	74.0 $\pm$ 6.9	19.2 $\pm$ 9.4		
Karikari et al. (2020) [45]	CN (268)	51.1	73.5 $\pm$ 6.5	14.2 $\pm$ 9.0	1, 2 and 3	Simoa - Karikari method
	AD (137)	52.2	73.4 $\pm$ 8.2	25.8 $\pm$ 8.6		
Mielke et al. (2018) [90]	CN (172)	69.2	71.9 $\pm$ 9.5	6.4 $\pm$ 6.4	1, 2 and 3	MSD
	AD (40)	23	67.7 $\pm$ 9.2	11.6 $\pm$ 4.1		
Thijssen et al. (2020) [89]	CN (69)	53.6	60.8 $\pm$ 22	2.4 $\pm$ 3	1, 2 and 3	MSD
	AD (56)	41.1	65 $\pm$ 9	8.4 $\pm$ 4		
Janelidze et al. (2020) - Cohort1 [90]	CN (26)	38.5	74 $\pm$ 5.2	1.3 $\pm$ 1.1	1, 2 and 3	MSD
	AD (38)	44.7	73 $\pm$ 8.1	4.4 $\pm$ 2.3		
Janelidze et al. (2020) - Cohort2 [90]	CN (126)	61.9	71 $\pm$ 5.2	1.2 $\pm$ 0.59	1, 2 and 3	MSD
	AD (81)	43.2	73 $\pm$ 5.2	2.8 $\pm$ 2.07		
Janelidze et al. (2020) - Cohort3 [90]	CN (47)	59.6	83 $\pm$ 9.6	1.8 $\pm$ 1.04	1, 2 and 3	MSD
	AD (16)	75.0	83 $\pm$ 7.4	4 $\pm$ 2.07		

cohorts, were retrieved using the Simoa technology [8, 27–29, 31–44, 46–49, 51–66, 91–93]. Our results indicated that the average plasma tau level in healthy populations was 3.07 pg/ml (95% CI 2.72–3.41, $I^2 = 99.7\%$, $P < 0.0001$). The plasma tau levels in the young, middle-aged, and old groups were 2.96 pg/ml (95% CI 2.20–3.72), 2.85 pg/ml (95% CI 2.22–3.47), and 3.23 pg/ml (95% CI 2.84–3.62), respectively (Fig. 2a), without significant differences ($P = 0.099$). However, significant differences were identified in the subgroup analysis by sex ratio (4.15 pg/ml vs 2.64 pg/ml vs 3.24 pg/ml; $P < 0.0001$; Fig. S2). Fifteen publications [67–81] reporting 20 cohorts were retrieved using IMR and two publications [82, 83] reporting four cohorts were

retrieved using a-EIMAF. The effect sizes (ESs) for plasma tau levels were 16.30 pg/ml (95% CI 14.61–17.99, $I^2 = 92.7\%$, $P < 0.0001$, Fig. S3) and 76.14 fg/ml (95% CI 72.34–79.93, $I^2 = 88.8\%$, $P < 0.0001$, Fig. S4), respectively.

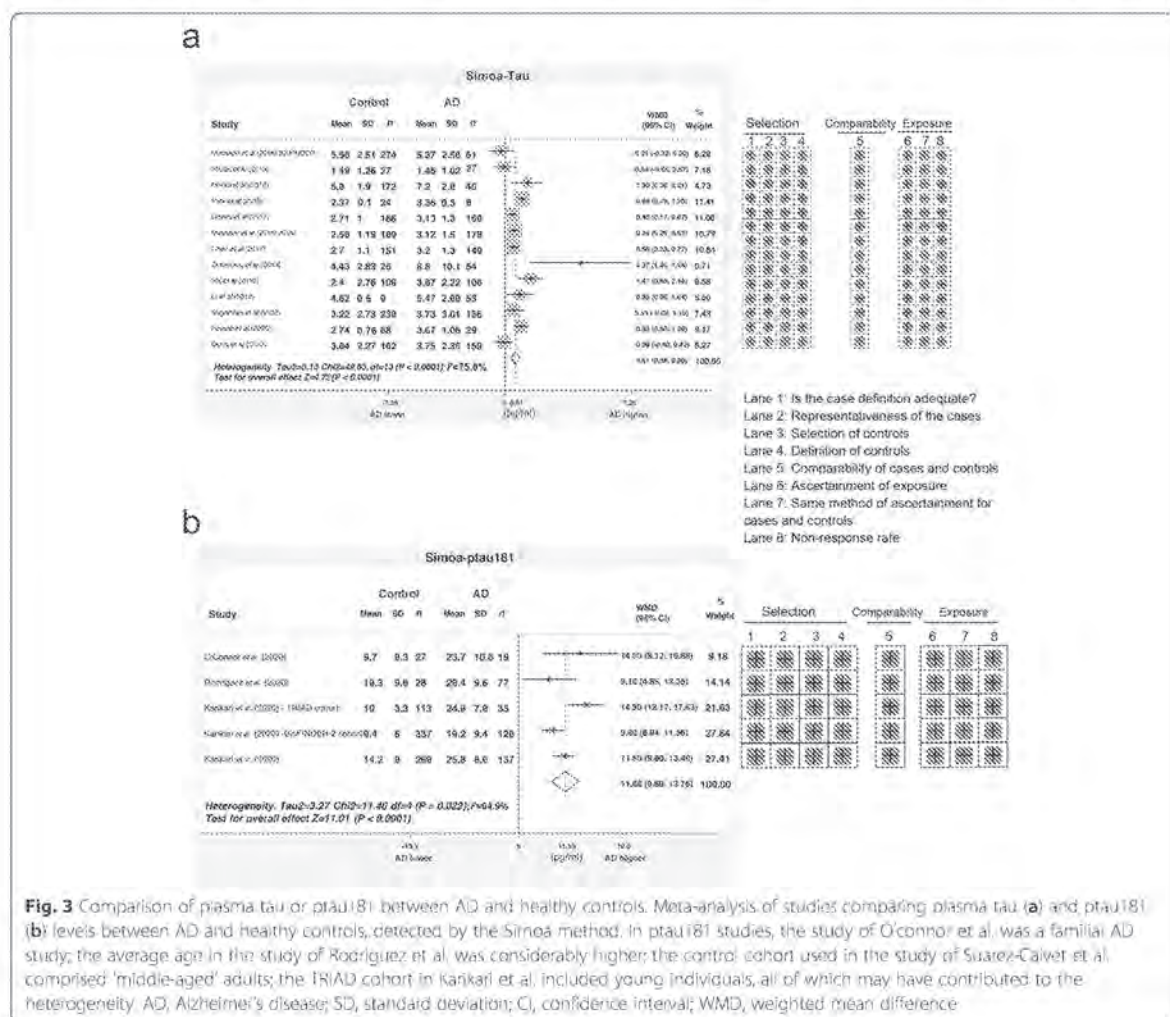
Nine studies that used Simoa or MSD methods were included for the analysis of normal range of plasma ptau181. Six publications, reporting seven cohorts, were retrieved using the Simoa technology [45, 84–88], including 1424 healthy subjects. The ES for plasma ptau181 levels in healthy populations was 11.18 pg/ml (95% CI 9.68–12.68, $I^2 = 95.9\%$, $P < 0.0001$, Fig. 2b). There were no significant differences in plasma ptau181 level in the subgroup analysis concerning age ($P = 0.181$)



or the sex ratio ($P = 0.168$, Fig. S2). Three publications [60, 89, 90] reporting five cohorts using the MSD method, including 440 healthy subjects who could not be grouped by age or sex ratio, were identified. The ES for plasma ptau181 levels measured by MSD was 2.48 pg/ml (95% CI 1.57–3.37, $I^2 = 97.0\%$, $P < 0.0001$; Fig. S4).

Plasma total tau and ptau181 in AD patients and controls
We then compared plasma tau and ptau181 between AD and controls. The random-effects model of meta-analysis with subgroup analysis was performed according to age and sex ratio. Among the 66 studies, 22 studies that used Simoa and IMR were included for the analysis of difference of plasma tau between AD and controls, and the number of studies that used other technologies was insufficient for analysis. For the Simoa method, 13 papers, reporting 14 cohorts [44, 55–66], were retrieved with a total of 1189 AD patients with 1611 controls. Our

analysis revealed a significantly higher plasma tau level in patients with AD, with an average WMD value of 0.61 (95% CI 0.36–0.86, $I^2 = 75.8\%$, $P < 0.0001$; Fig. 3a). The WMDs for subgroups divided by different male compositions were 0.48 (< 40% male, 95% CI -0.18–1.14), 0.99 (40%–60% male, 95% CI 0.54–1.44) and 1.30 (> 60% male, 95% CI 0.39–2.21), respectively, indicating a strong effect of sex on plasma tau levels in AD patients (Fig. S5). Meta-regression analysis identified that only the sample size significantly contributed to the high heterogeneity ($P = 0.027$, Table S2). The funnel plots and the Egger's test suggested no publication bias ($P = 0.133$) (Fig. S6). Similarly, 9 papers [72–74, 76–81] reporting 11 cohorts using the IMR method (362 controls and 267 patients) were identified (Fig. S5), showing an average WMD value of 24.83 (95% CI 15.70–33.96, $I^2 = 98.9\%$, $P < 0.0001$). There was no publication bias ($P = 0.175$, Fig. S6), but



the year of publication ($P = 0.013$) may influence the heterogeneity (Table S2).

Seven studies that used Simoa and MSD were included for the analysis of the plasma ptau181 difference between AD and controls. Four publications reporting five cohorts [45, 86–88] using the Simoa technology were retrieved, resulting in a total of 773 controls and 392 patients. The plasma ptau181 levels were significantly elevated in AD patients, with an average WMD value of 11.68 (95% CI 9.60–13.76, $I^2 = 64.9\%$, $P < 0.0001$, Fig. 3b). The funnel plots suggested no publication bias for plasma ptau181 ($P = 0.635$, Fig. S6). Similar results were found based on three papers reporting five cohorts [60, 89, 90] using the MSD method (440 controls and 231 patients), with a WMD of 3.53 (95% CI 1.97–5.09, $I^2 = 93.0\%$, $P < 0.0001$, Fig. S5). There was also no publication bias ($P = 0.055$, Fig. S6).

The diagnostic accuracy of plasma tau/ptau181 for AD

Among the 66 studies, four studies reporting five cohorts [44, 61, 64, 65] were pooled for meta-analysis of diagnostic accuracy of plasma tau using the Simoa method. The estimate values of diagnostic accuracy are summarized in Table S3. The pooled sensitivity and specificity of plasma tau to predict AD were 0.75 (95% CI 0.60–0.86) and 0.69 (95% CI 0.57–0.79), respectively (Fig. 4a). The DOR was 6.16 (95% CI 3.02–12.53), and the AUC of the HSROC curve was 0.77 (95% CI 0.74–0.81) (Fig. 4b). Fagan's nomogram showed that the probability of AD increased from 25% (pre-test probability) to 45% (post-test probability) when plasma tau level was above the threshold, while the probability of AD decreased to as low as 11% when plasma tau level was below the threshold (Fig. 4c).

Four studies using the Simoa method reporting five cohorts [45, 86–88] were pooled for meta-analysis to test the diagnostic accuracy of plasma ptau181. The estimate values of diagnostic accuracy are summarized in Table S3. The pooled sensitivity and specificity of ptau181 were 0.89 (95% CI 0.81–0.93) and 0.86 (95% CI 0.79–0.91), respectively (Fig. 4d). The DOR was 46 (95% CI 18–123), and the AUC of the HSROC curve was 0.93 (95% CI 0.91–0.95) (Fig. 4e). The probability of AD increased from 25% (pre-test probability) to 67% (post-test probability) when plasma ptau181 level was above the threshold and decreased to as low as 4% when plasma ptau181 level below the threshold (Fig. 4f). Consistently, two studies using the MSD method reporting four cohorts [89, 90] were pooled for meta-analysis. The estimate values of diagnostic accuracy are summarized in Table S3. The pooled sensitivity of ptau181 was 0.87 (95% CI 0.78–0.92), and the pooled specificity was 0.79 (95% CI 0.73–0.83, Fig. S7). The DOR was 23.98 (95% CI 10.14–56.69), and the AUC of the HSROC curve was 0.86 (95% CI 0.83–0.89, Fig. S7). The

probability of AD increased from 25% (pre-test probability) to 58% (post-test probability) when plasma ptau181 level was above the threshold, and decreased to 5% when plasma ptau181 level was below the threshold (Fig. S7).

Discussion

With a growing interest in plasma tau detection during the last 10 years, heterogeneity between studies has been consistently presented. Besides, given the inconsistency between publications regarding the plasma tau levels in AD (compared to control), it is difficult to determine the suitability of plasma tau/ptau181 to predict AD. In this meta-analysis, we found that both plasma tau and ptau181 have diagnostic values, and both of them are significantly higher in AD patients than in controls. We also established the average plasma tau and ptau181 levels based on the current literature, which may be used as a reference point in future research.

Currently, there are four ultrasensitive assays that can be used for plasma tau and ptau detection. In this meta-analysis, we examined them independently. Despite the differences in absolute values of plasma tau and ptau, results from all assays support the notion that both plasma tau and ptau181 are elevated in AD compared with healthy controls. Based on the available publications of each method, we have been able to calculate the sensitivity and diagnostic accuracy for AD of studies using Simoa (tau AUC: 0.77; ptau181 AUC: 0.93), and MSD methods (ptau181 AUC: 0.86), while the other two methods had limited applications in AD research.

Both A β and tau can now be visualized in the brain using PET and be measured in the CSF. Although medical history and cognitive and neurologic examinations remain the most important diagnostic tool in the clinic [94], these new techniques can assist the diagnosis of AD [1]. However, the associated cost and infrastructure requirements have limited their use, especially in developing countries. It is critical to accurately measure AD-associated proteins in plasma and determine their relationships with brain and CSF contents. Proteins in plasma may reflect protein levels in the brain and CSF, especially in the state of illness. For example, the correlation between CSF and plasma ptau181 is significant in PET A β -positive cases, even without cognitive impairment [90]. Higher ptau181 is associated with increased standardized uptake value ratio of tau PET in Braak I–IV regions of interest [89]. Furthermore, our meta-analysis results reflected the high diagnostic accuracy of plasma tau (AUC 0.77, 95% CI 0.74–0.81) and ptau181 (AUC 0.93, 95% CI 0.91–0.95), similar to the diagnostic accuracy of tau PET (AUC 0.98, 95% CI 0.94–1.00) and CSF ptau181 (AUC 0.97, 95% CI 0.92–1.00) [90]. In contrast, there is only a weak association between CSF and plasma tau, as confirmed by two independent studies

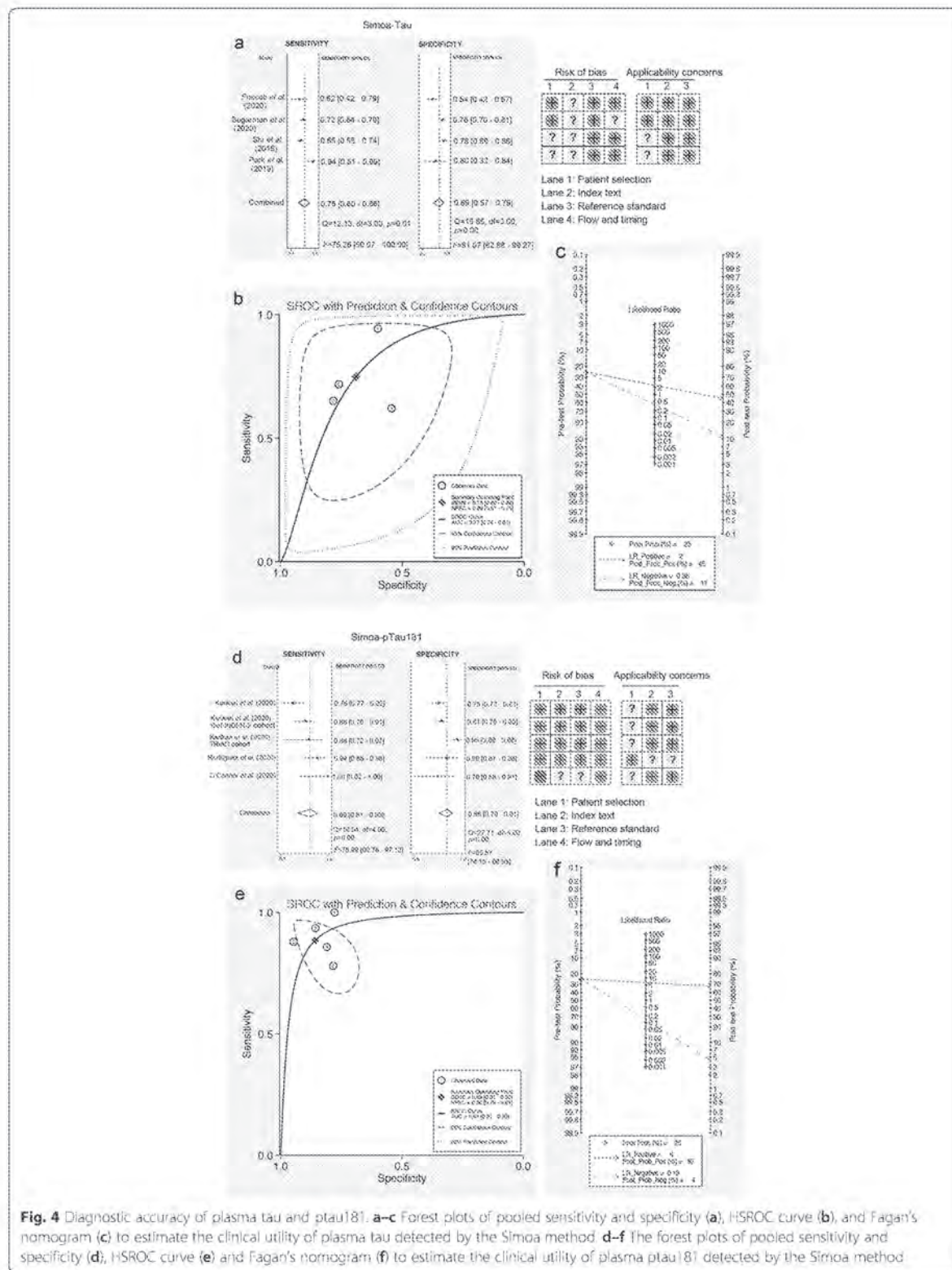


Fig. 4 Diagnostic accuracy of plasma tau and ptau181. **a–c** Forest plots of pooled sensitivity and specificity (**a**), HSROC curve (**b**), and Fagan's nomogram (**c**) to estimate the clinical utility of plasma tau detected by the Simoa method. **d–f** The forest plots of pooled sensitivity and specificity (**d**), HSROC curve (**e**) and Fagan's nomogram (**f**) to estimate the clinical utility of plasma ptau181 detected by the Simoa method

[46, 65], suggesting that they may go through different metabolism. There are no significant differences in plasma or serum A β between AD and controls, and plasma A β may reflect peripheral A β generation instead of brain pathology [95].

In addition, the current meta-analysis supports plasma ptau181 as a better predictive biomarker than plasma tau for AD. The difference in plasma ptau181 between AD and controls was greater than plasma tau in our analysis (WMD: 11.68 pg/ml vs 0.83 pg/ml), and the pooled diagnostic accuracy of ptau181 was also higher (AUC: 0.93 vs 0.77). These are consistent with a previous report that the plasma ptau181 is more strongly associated with both A β and tau PET than plasma tau [60], and can differentiate AD from non-AD pathologies with high accuracy (AUC 97.4, 95% CI 94.1–100%) eight years before death [87], collectively highlighting the potential of ptau181 as a biomarker for AD pathology.

We also found a higher level of plasma ptau181 compared to total tau in the meta-analysis. Phosphorylation is a post-translational modification of the protein, and theoretically, ptau181 should be a portion of total tau in any given tissue [96]. However, according to our analysis of publications based on the Simoa method, the mean level of ptau181 was higher than total tau (11.18 pg/ml vs 3.07 pg/ml). There could be differences in calibration standard, and at this stage, it is not feasible to compare the calculated values of tau and ptau181.

Limitations

There were some limitations in this systematic review and meta-analysis. The ultrasensitive measurement of plasma tau has yet to be extensively tested, leading to high heterogeneity in the results. Meta-regression analyses suggested that the heterogeneity was partly explained by the sample size of studies included. In addition, most of the studies included in the current analysis used clinical diagnostic guidelines rather than gold-standard autopsy-confirmed AD, which can induce heterogeneity. One study [57] on neuropathologically confirmed autopsy cases has revealed a greater change in plasma tau (WMD 5.81), compared to the results from the clinically diagnosed cases (WMD 0.83), highlighting the potential error induced by diagnosis. Even the diagnosis is correct, AD itself can be heterogeneous. For example, familial cohorts [86] may lead to different results from sporadic cohorts.

On the other hand, due to the lack of information on ApoE4 status, we could not measure the impact of ApoE on plasma tau and ptau, leading to potential heterogeneity. We have considered age as a factor for heterogeneity, but due to the limited data, we can only analyze the effect of age in healthy populations, where we found no effect of age on plasma tau. Future analysis should be performed when there are more studies on early-onset AD.

We excluded a few publications for analysis. Two using the Simoa platform were excluded as the methods used in those studies had not been validated in multiple cohorts or studies [97], or were specifically validated for CSF (not plasma) [61]. Although results of the two studies are consistent with our findings, it is challenging to combine them with those using the commercialized plasma tau detection kit developed by Quanterix. A few other studies were focused on the serum tau or ptau [98, 99], and were also excluded from our analysis.

We have to mention that some studies on ptau181 were recently published during the final revision of the manuscript, and were not included. These latest studies have investigated the dynamic changes of plasma ptau181 across the AD spectrum [100] and the relationship between polygenic risk scores for AD and plasma ptau181 [101]. They have also compared performance of ptau181 with other biomarkers in AD and MCI prediction [102] or amyloid PET status prediction [103]. In addition, ptau217 [104] and ptau231 (Ashton et al. in press) have been reported recently to differentiate AD from other neurodegenerative disorders and be associated with tau pathology in the brain. The plasma ptau217 may out-perform ptau181 with a higher AUC and stronger correlations with the tau PET [9, 104]. However, here we did not include plasma ptau217 and ptau231 studies in our meta-analysis, due to the limited number of publications.

Conclusion

In summary, the results presented provide preliminary evidence of plasma tau and its phosphorylated form ptau181 as potential biomarkers for neurological diseases, especially for AD diagnosis, which may facilitate drug discovery of these diseases by selecting correct patients for clinical trials.

Abbreviations

AD: Alzheimer's disease; ptau181: Phosphorylated tau 181; AUC: Area under curve; ROC: Receiver operating characteristic; NIA-AA: National Institute on Aging—Alzheimer's Association; A β : Amyloid-beta; PET: Positron emission tomography; NFT: Neurofibrillary tangle; CSF: Cerebrospinal fluid; Simoa: Single-molecule Array; IMR: ImmunoMagnetic Reduction; a-EIMA: E: multi-arrayed fiber optics conjugated with rolling circle amplification; MSD: Meso Scale Discovery; NOS: Newcastle-Ottawa Scale; QUADAS-2: Quality Assessment of Diagnostic Accuracy Studies-2 tool; WMD: Weighted mean difference; DOR: Diagnostic odds ratio; HSROC: Hierarchical summary receiver operating characteristic

Additional Files

Additional file 1 Table S1. The Newcastle-Ottawa Scale (NOS) for assessing the quality of studies in meta-analyses. **Table S2.** Meta-regression results. **Table S3.** Summary of estimate values of diagnostic accuracy. **Additional file 2 Fig. S1.** Quality assessment results of included articles. **Fig. S2.** The levels of plasma tau and ptau181 in healthy people using

Simoa in different subgroups. **Fig. S3.** The level of plasma tau in healthy people using IMR in different subgroups. **Fig. S4.** The levels of plasma tau and ptau181 in healthy people using EIMAF/a-EIMAF and MSD. **Fig. S5.** Comparison of plasma tau and ptau181 between AD and healthy controls using Simoa, IMR, and MSD. **Fig. S6.** Funnel plot of the random-effect analysis. **Fig. S7.** The diagnostic accuracy of plasma ptau181 using MSD.

Acknowledgments

Not applicable.

Authors' contributions

PI. contributed to the conception, project design, and data interpretation. XLD and STZ performed literature research and drafted the manuscript. LW and LJ helped to collect the data and performed statistical analyses. TJ contributed to data interpretation. All authors edited and approved the manuscript.

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and its additional files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

- Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement*. 2018;14(4):535–62.
- Guo T, Zhang D, Zeng Y, Huang TY, Xu H, Zhao Y. Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's disease. *Mol Neurodegener*. 2020;15(1):40.
- Lei P, Ayton S, Finkelstein DI, Spoerri L, Ciccostato GD, Wright DK, et al. Tau deficiency induces parkinsonism with dementia by impairing APP-mediated iron export. *Nat Med*. 2012;18(2):291–5.
- Lei P, Ayton S, Moon S, Zhang Q, Volitakis I, Finkelstein DI, et al. Motor and cognitive deficits in aged tau knockout mice in two background strains. *Mol Neurodegener*. 2014;9(1):29.
- Lei P, Ayton S, Appukuttan AT, Moon S, Duce JA, Volitakis I, et al. Lithium suppression of tau induces brain iron accumulation and neurodegeneration. *Mol Psychiatry*. 2017;22(3):396–406.
- Tuo QZ, Lei P, Jackman KA, Li XL, Xiong H, Li XL, et al. Tau-mediated iron export prevents ferroptotic damage after ischemic stroke. *Mol Psychiatry*. 2017;22(11):1520–30.
- Shen LL, Manuvas-Tan NB, Gao SH, Li WW, Zeng F, Zhu C, et al. The ProNGF/p75NTR pathway induces tau pathology and is a therapeutic target for FTD-tau. *Mol Psychiatry*. 2018;23(8):1813–24.
- Dage JL, Wennberg AMV, Airey DC, Hagen CE, Knopman DS, Machulda MW, et al. Levels of tau protein in plasma are associated with neurodegeneration and cognitive function in a population-based elderly cohort. *Alzheimers Dement*. 2016;12(12):1226–34.
- Janelidze S, Simonrud E, Smith R, Palmqvist S, Mattsson N, Airey DC, et al. Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. *Nat Commun*. 2020;11(1):1683.
- Barthélemy NR, Li Y, Joseph Mathurin N, Gordon BA, Hassenstab J, Benzinger TLS, et al. A soluble phosphorylated tau signature links tau, amyloid and the evolution of stages of dominantly inherited Alzheimer's disease. *Nat Med*. 2020;26(3):398–407.
- Kankan TK, Emeršić A, Villon A, Lenero-Rodriguez J, Ashton NJ, Kramberger MG, et al. Head-to-head comparison of clinical performance of CSF phospho-tau T181 and T217 biomarkers for Alzheimer's disease diagnosis. *Alzheimers Dement*. 2020. <https://doi.org/10.1002/alz.12236>
- Meng J, Lei P. Plasma pTau181 as a biomarker for Alzheimer's disease. *MedComm*. 2020;1(1):74–6.
- Rissin DM, Kan CW, Campbell TG, Howes SC, Fournier DR, Song L, et al. Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. *Nat Biotechnol*. 2010;28(6):595–9.
- Hong CY, Wu CC, Chiu YC, Yang SY, Horng HF, Yang HC. Magnetic susceptibility reduction method for magnetically labeled immunoassay. *Appl Phys Lett*. 2006;88(21):62.
- Rubenstein R, Chang B, Davies P, Wagner AK, Robertson CS, Wang KK. A novel, ultrasensitive assay for tau: potential for assessing traumatic brain injury in tissues and biofluids. *J Neurotrauma*. 2015;32(5):342–52.
- Zu YB, Bard AJ. Electrogenerated chemiluminescence. 66. The role of direct coreactant oxidation in the ruthenium tris (2,2'-bipyridyl)/tripropylamine system and the effect of halide ions on the emission intensity. *Anal Chem*. 2000;72(14):3223–32.
- Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. 2009;6(7):e1000097.
- McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kowas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;7(3):263–9.
- McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984;34(7):939–44.
- Wells GA, Shea B, O'Connell D, Peterson J, Welch V, et al. The Newcastle-Ottawa scale (NOS) for assessing the quality of non-randomised studies in meta-analyses. In: 3rd Symposium on Systematic Reviews: Beyond the Basics. July 3–5. 2000. Oxford; 2000.
- van Houwelingen HC, Arends LR, Stijnen T. Advanced methods in meta-analysis: multivariate approach and meta-regression. *Stat Med*. 2002;21(4):589–624.
- Harbord RM, Whiting P. Metandi: meta-analysis of diagnostic accuracy using hierarchical logistic regression. *Stata J*. 2009;9(2):211–29.
- Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med*. 2002;21(11):1539–58.
- Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ*. 2003;327(7414):557–60.
- Sterne JA. Meta-analysis in Stata: an updated collection from the Stata journal. *StataCorp LP*; 2009.

26. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med*. 2011;155(8):529–36.
27. Shahim P, Tegner Y, Wilson DH, Randall J, Skillback Y, Pazonki D, et al. Blood biomarkers for brain injury in concussed professional ice hockey players. *JAMA Neurol*. 2014;71(6):684–92.
28. Bogoslovsky T, Wilson D, Chen Y, Hanlon D, Gill J, Jeromin A, et al. Increases of plasma levels of glial fibrillary acidic protein, tau, and amyloid beta up to 90 days after traumatic brain injury. *J Neurotrauma*. 2017;34(1):66–73.
29. Olivera A, Lejbman N, Jeromin A, French LM, Kim HS, Cashion A, et al. Peripheral total tau in military personnel who sustain traumatic brain injuries during deployment. *JAMA Neurol*. 2015;72(10):1109–16.
30. Oliver JM, Jones MT, Anzalone AJ, Kirk KM, Gable DA, Repshas JT, et al. A season of American football is not associated with changes in plasma tau. *J Neurotrauma*. 2017;34(23):3295–300.
31. Alosco ML, Tripodis Y, Jamagin J, Baugh CM, Martin B, Chaisson CE, et al. Repetitive head impact exposure and later life plasma total tau in former National Football League players. *Alzheimers Dement (Amst)*. 2016;7:33–40.
32. Mielke MM, Hagen CE, Wennberg AMV, Airey DC, Savica R, Knopman DS, et al. Association of plasma total tau level with cognitive decline and risk of mild cognitive impairment or dementia in the Mayo Clinic study on aging. *JAMA Neurol*. 2017;74(9):1073–80.
33. Müller S, Preische O, Gopfert JC, Yanez VAC, Joos TO, Boecker H, et al. Tau plasma levels in subjective cognitive decline: results from the DELCODE study. *Sci Rep*. 2017;7(1):9529.
34. Kasai T, Tatebe H, Kondo M, Ishii R, Ohmichi T, Yeung WTE, et al. Increased levels of plasma total tau in adult Down syndrome. *PLoS One*. 2017;12(11):e0188802.
35. Foiani MS, Woolfcott IG, Heiler C, Bocchetta M, Heslegrave A, Dick KM, et al. Plasma tau is increased in frontotemporal dementia. *J Neurol Neurosurg Psychiatry*. 2018;89(8):804–7.
36. Bergman L, Zetterberg H, Kaihola H, Hagberg H, Blennow K, Akerud H. Blood-based cerebral biomarkers in preeclampsia: plasma concentrations of NFL, tau, S100B andNSE during pregnancy in women who later develop preeclampsia – a nested case control study. *PLoS One*. 2018;13(5):e0196025.
37. Lippa SM, Yeh PH, Gill J, French LM, Brickell TA, Lange RT. Plasma tau and amyloid are not reliably related to injury characteristics, neuropsychological performance, or white matter integrity in service members with a history of traumatic brain injury. *J Neurotrauma*. 2019;36(14):2190–9.
38. Verberk IMW, Slot RE, Verfaillie SCJ, Heijst H, Prins ND, van Berckel BNM, et al. Plasma amyloid as prescreen for the earliest Alzheimer pathological changes. *Ann Neurol*. 2018;84(5):648–58.
39. Shahim P, Tegner Y, Marklund N, Blennow K, Zetterberg H. Neurofilament light and tau as blood biomarkers for sports-related concussion. *Neurology*. 2018;90(20):e1780–8.
40. Wallace C, Zetterberg H, Blennow K, van Donkelaar P. No change in plasma tau and serum neurofilament light concentrations in adolescent athletes following sport-related concussion. *PLoS One*. 2018;13(10):e0206466.
41. Motamed V, Karelisky R, Matsangas P, Mithani S, Jeromin A, Brock MS, et al. Elevated tau and interleukin-6 concentrations in adults with obstructive sleep apnea. *Sleep Med*. 2018;43:71–6.
42. Fortea J, Carmona-Iragui M, Benítez J, Fernández S, Videla L, Barroeta I, et al. Plasma and CSF biomarkers for the diagnosis of Alzheimer's disease in adults with Down syndrome: a cross-sectional study. *Lancet Neurol*. 2018;17(10):860–9.
43. Zeitlberger AM, Thomas-Black G, García-Moreno H, Foiani M, Heslegrave AJ, Zetterberg H, et al. Plasma markers of neurodegeneration are raised in Friedreich's ataxia. *Front Cell Neurosci*. 2018;12:366.
44. Shi M, Kovac A, Korff A, Cook TJ, Ginghina C, Bullock RM, et al. CNS tau efflux via exosomes is likely increased in Parkinson's disease but not in Alzheimer's disease. *Alzheimers Dement*. 2016;12(11):1125–31.
45. Karikari TK, Benedet AL, Ashton NJ, Lantero-Rodriguez J, Snellman A, Suarez-Calvet M, et al. Diagnostic performance and prediction of clinical progression of plasma phospho-tau181 in the Alzheimer's disease neuroimaging initiative. *Mol Psychiatry*. 2021;26(2):429–42.
46. Pase MP, Beiser AS, Himali JJ, Satizabal CL, Aparicio HJ, DeCarli C, et al. Assessment of plasma total tau level as a predictive biomarker for dementia and related endophenotypes. *JAMA Neurol*. 2019;76(5):598.
47. Korley FK, Yue JK, Wilson DH, Hrusovsky K, Diaz-Arastia R, Ferguson AR, et al. Performance evaluation of a multiplex assay for simultaneous detection of four clinically relevant traumatic brain injury biomarkers. *J Neurotrauma*. 2018;36(1):182–7.
48. Kritikos M, Clouston SAP, Diminich ED, Deri Y, Yang X, Carr M, et al. Pathway analysis for plasma β -amyloid, tau and neurofilament light (ATN) in world trade center responders at midlife. *Neurol Ther*. 2020;9(1):159–71.
49. de Wolf F, Ghanbari M, Licher S, McRae-McKee K, Gras L, Weverling GJ, et al. Plasma tau, neurofilament light chain and amyloid- β levels and risk of dementia: a population-based cohort study. *Brain*. 2020;143(4):1220–32.
50. Verberk IMW, Hendriksen HMA, van Harten AC, Wessdman LMP, Verfaillie SCJ, van den Bosch KA, et al. Plasma amyloid is associated with the rate of cognitive decline in cognitively normal elderly: the SCIENCE project. *Neurobiol Aging*. 2020;89:99–107.
51. Patinson CL, Gill JM, Lippa SM, Brickell TA, French LM, Lange RT. Concurrent mild traumatic brain injury and posttraumatic stress disorder is associated with elevated tau concentrations in peripheral blood plasma. *J Trauma Stress*. 2019;32(4):546–54.
52. Romero JR, Demisse S, Beiser A, Himali JJ, DeCarli C, Levy D, et al. Relation of plasma β -amyloid, clusterin, and tau with cerebral microbleeds: Framingham heart study. *Ann Clin Transl Neurol*. 2020;7(7):1083–91.
53. Petersen ME, Rafii MS, Zhang F, Hall J, Julovich D, Ances BM, et al. Plasma total-tau and neurofilament light chain as diagnostic biomarkers of Alzheimer's disease dementia and mild cognitive impairment in adults with Down syndrome. *J Alzheimers Dis*. 2021;79(2):671–81.
54. Cantero JL, Atienza M, Ramos-Cejudo J, Fossati S, Wisniewski T, Osorio RS. Plasma tau predicts cerebral vulnerability in aging. *Aging*. 2020;12(21):21004–22.
55. Zetterberg H, Wilson D, Andreasson U, Minthon L, Blennow K, Randall J, et al. Plasma tau levels in Alzheimer's disease. *Alzheimers Res Ther*. 2013;5(2):9.
56. Mattsson N, Zetterberg H, Janelidze S, Insel PS, Andreasson U, Stomrud E, et al. Plasma tau in Alzheimer disease. *Neurology*. 2016;87(17):1827–35.
57. Kovacs GG, Andreasson U, Liman V, Regelsberger G, Lutz MJ, Danics K, et al. Plasma and cerebrospinal fluid tau and neurofilament concentrations in rapidly progressive neurological syndromes: a neuropathology-based cohort. *Eur J Neurol*. 2017;24(11):1326–e77.
58. Deters KD, Risacher SL, Kim S, Nho K, West JD, Blennow K, et al. Plasma tau association with brain atrophy in mild cognitive impairment and Alzheimer's disease. *J Alzheimers Dis*. 2017;58(4):1245–54.
59. Chen J, Yu JJ, Wojta K, Wang HF, Zetterberg H, Blennow K, et al. Genome-wide association study identifies MAPT locus influencing human plasma tau levels. *Neurology*. 2017;88(7):669–76.
60. Mielke MM, Hagen CE, Xu J, Chai X, Wenn P, Lowe VJ, et al. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. *Alzheimers Dement*. 2018;14(8):989–97.
61. Park JC, Han SH, Yi D, Byun MS, Lee JH, Jang S, et al. Plasma tau/amyloid-beta1–42 ratio predicts brain tau deposition and neurodegeneration in Alzheimer's disease. *Brain*. 2019;142(3):771–86.
62. Li WW, Shen YY, Tian DY, Bu XL, Zeng F, Liu YH, et al. Brain amyloid-beta deposition and blood biomarkers in patients with clinically diagnosed Alzheimer's disease. *J Alzheimers Dis*. 2019;69(1):169–78.
63. Starin CM, Ashton NJ, Hamburg S, Hithersay R, Wiseman FK, Mok KY, et al. Plasma biomarkers for amyloid, tau, and cytokines in Down syndrome and sporadic Alzheimer's disease. *Alzheimers Res Ther*. 2019;11(1):26.
64. Sugamari MA, Zetterberg H, Blennow K, Tripodis Y, McKee AC, Stein TD, et al. A longitudinal examination of plasma neurofilament light and total tau for the clinical detection and monitoring of Alzheimer's disease. *Neurobiol Aging*. 2020;94:60–70.
65. Fossati S, Ramos-Cejudo J, Debure L, Piraglia E, Sone JY, Li Y, et al. Plasma tau complements CSF tau and P-tau in the diagnosis of Alzheimer's disease. *Alzheimers Dement (Amst)*. 2019;11:483–92.
66. Deniz K, Ho CCG, Malphrus KG, Reddy JS, Nguyen T, Carnwath TP, et al. Plasma biomarkers of Alzheimer's disease in African Americans. *J Alzheimers Dis*. 2021;79(1):323–34.
67. Lin CH, Yang SY, Horng HE, Yang CC, Chieh JJ, Chen HH, et al. Plasma biomarkers differentiate Parkinson's disease from atypical parkinsonism syndromes. *Front Aging Neurosci*. 2018;10:123.
68. Chi NF, Chao SP, Huang UK, Chan L, Chen YR, Chiou HY, et al. Plasma amyloid beta and tau levels are predictors of post-stroke cognitive impairment: a longitudinal study. *Front Neurol*. 2019;10:715.
69. Chen TB, Lee YJ, Lin SY, Chen JP, Hu CJ, Wang PN, et al. Plasma A β 42 and total tau predict cognitive decline in amnesic mild cognitive impairment. *Sci Rep*. 2019;9(1):13984.

70. Chen NC, Chen HL, Li SH, Chang YH, Chen MH, Tsai NW, et al. Plasma levels of α -synuclein, A β -40 and τ -tau as biomarkers to predict cognitive impairment in Parkinson's disease. *Front Aging Neurosci.* 2020;12:112.
71. Fang WQ, Hwu WL, Chien YH, Yang SY, Chieh JJ, Chang LM, et al. Composite scores of plasma tau and β -amyloids correlate with dementia in Down syndrome. *ACS Chem Neurosci.* 2020;11(2):191–6.
72. Chiu MJ, Fan LY, Chen TF, Chen YF, Chieh JJ, Horng HE. Plasma tau levels in cognitively normal middle-aged and older adults. *Front Aging Neurosci.* 2017;9:51.
73. Chiu MJ, Chen YF, Chen TF, Yang SY, Yang FP, Tseng TW, et al. Plasma tau as a window to the brain: negative associations with brain volume and memory function in mild cognitive impairment and early Alzheimer's disease. *Hum Brain Mapp.* 2014;35(7):3132–42.
74. Tzen KY, Yang SY, Chen TF, Cheng TW, Horng HE, Wen HP, et al. Plasma A β but not tau is related to brain PIB retention in early Alzheimer's disease. *ACS Chem Neurosci.* 2014;5(9):830–6.
75. Yang SY, Chiu MJ, Chen TF, Lin CH, Jeng JS, Tang SC, et al. Analytical performance of reagent for assaying tau protein in human plasma and feasibility study screening neurodegenerative diseases. *Sci Rep.* 2017;7(1):9304.
76. Lee NC, Yang SY, Chieh JJ, Huang PT, Chang LM, Chiu YN, et al. Blood beta-amyloid and tau in Down syndrome: a comparison with Alzheimer's disease. *Front Aging Neurosci.* 2016;8:316.
77. Lue LF, Sabbagh MN, Chiu MJ, Jing N, Snyder NL, Schmitz C, et al. Plasma levels of A β 42 and tau identified probable Alzheimer's dementia: findings in two cohorts. *Front Aging Neurosci.* 2017;9:226.
78. Yang CC, Chiu MJ, Chen TF, Chang HL, Liu BH, Yang SY. Assay of plasma phosphorylated tau protein (threonine 181) and total tau protein in early-stage Alzheimer's disease. *J Alzheimers Dis.* 2018;61(4):1323–32.
79. Chiu MJ, Lue LF, Sabbagh MN, Chen TF, Chen HH, Yang SY. Long-term storage effects on stability of A β 40, A β 42, and total tau proteins in human plasma samples measured with immunomagnetic reduction assays. *Dement Geriatr Cogn Dis Extra.* 2019;9(1):77–86.
80. Jiao F, Yi F, Wang Y, Zhang S, Guo Y, Du W, et al. The validation of multifactor model of plasma A β (42) and total-tau in combination with MoCA for diagnosing probable Alzheimer disease. *Front Aging Neurosci.* 2020;12:212.
81. Liu HC, Chiu MJ, Lin CH, Yang SY. Stability of plasma amyloid- β 1–40, amyloid- β 1–42, and total tau protein over repeated freeze/thaw cycles. *Dement Geriatr Cogn Dis Extra.* 2020;10(1):46–55.
82. Rubenstein R, Chang B, Yue JK, Chiu A, Winkler EA, Puccio AM, et al. Comparing plasma phospho tau, total tau, and phospho tau-total tau ratio as acute and chronic traumatic brain injury biomarkers. *JAMA Neurol.* 2017;74(9):1063–72.
83. Gardner RC, Rubenstein R, Wang KKW, Korley FK, Yue JK, Yuh EL, et al. Age-related differences in diagnostic accuracy of plasma glial fibrillary acidic protein and tau for identifying acute intracranial trauma on computed tomography: a TRACK-TBI study. *J Neurotrauma.* 2018;35(20):2341–50.
84. Suárez-Calvet M, Karikari TK, Ashton NJ, Lantero Rodríguez J, Millá-Alomà M, Gisbert JD, et al. Novel tau biomarkers phosphorylated at T181, T217 or T231 rise in the initial stages of the preclinical Alzheimer's continuum when only subtle changes in A β pathology are detected. *EMBO Mol Med.* 2020;12(12):e12921.
85. Moscoso A, Grothe MJ, Ashton NJ, Karikari TK, Lantero Rodríguez J, Snellman A, et al. Longitudinal associations of blood phosphorylated Tau181 and neurofilament light chain with neurodegeneration in Alzheimer disease. *JAMA Neurol.* 2021. <https://doi.org/10.1001/jamaneurol.2020.4886>.
86. O'Connor A, Karikari TK, Poole T, Ashton NJ, Lantero Rodríguez J, Khalun A, et al. Plasma phospho-tau181 in presymptomatic and symptomatic familial Alzheimer's disease: a longitudinal cohort study. *Mol Psychiatry.* 2020. <https://doi.org/10.1038/s41380-020-0838-x>.
87. Lantero Rodríguez J, Karikari TK, Suárez-Calvet M, Troakes C, King A, Emersic A, et al. Plasma p-tau181 accurately predicts Alzheimer's disease pathology at least 8 years prior to post-mortem and improves the clinical characterisation of cognitive decline. *Acta Neuropathol.* 2020;140(3):267–78.
88. Karikari TK, Pascoal TA, Ashton NJ, Janelidze S, Benedet AL, Rodríguez JL, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol.* 2020;19(5):422–33.
89. Thissen EH, La Joie R, Wolf A, Suom A, Wang P, Iaccarino L, et al. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. *Nat Med.* 2020;26(3):387–97.
90. Janelidze S, Mattsson N, Palmqvist S, Smith R, Beach TG, Serrano GE, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat Med.* 2020;26(3):379–86.
91. Kitaguchi N, Tatebe H, Sakai K, Kawaguchi K, Matsunaga S, Kitajima T, et al. Influx of tau and amyloid-beta proteins into the blood during hemodialysis as a therapeutic extracorporeal blood amyloid-beta removal system for Alzheimer's disease. *J Alzheimers Dis.* 2019;69(3):687–707.
92. Oliver JM, Anzalone AJ, Stone JD, Turner SM, Bluetit D, Gamson JC, et al. Fluctuations in blood biomarkers of head trauma in NCAA football athletes over the course of a season. *J Neurosurg.* 2019;130(5):1655–62.
93. Shi Y, Lu X, Zhang L, Shu H, Gu L, Wang Z, et al. Potential value of plasma amyloid-beta, total tau, and neurofilament light for identification of early Alzheimer's disease. *ACS Chem Neurosci.* 2019;10(8):3479–85.
94. Arvanitakis Z, Shah RC, Bennett DA. Diagnosis and management of dementia: review. *JAMA.* 2019;322(16):1589–99.
95. Olsson B, Lautner R, Andreasson U, Ohlert A, Portelius E, Björke M, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol.* 2016;15(7):673–84.
96. Wang HY, Bakshi K, Frankfurt M, Stucky A, Guberhan M, Shah SM, et al. Reducing amyloid-related Alzheimer's disease pathogenesis by a small molecule targeting filamin A. *J Neurosci.* 2012;32(29):9773–84.
97. Tatebe H, Kasai T, Ohnishi T, Kishi Y, Kakeya T, Wamagai M, et al. Quantification of plasma phosphorylated tau to use as a biomarker for brain Alzheimer pathology: pilot case-control studies including patients with Alzheimer's disease and Down syndrome. *Mol Neurodegener.* 2017;12(1):63.
98. Benussi A, Karikari TK, Ashton NJ, Gazzina S, Premi E, Benussi L, et al. Diagnostic and prognostic value of serum NfL and p-tau (181) in frontotemporal lobar degeneration. *J Neurol Neurosurg Psychiatry.* 2020;91(9):960–7.
99. Shelkar S, Kumar R, Rai N, Kumar V, Singh K, Upadhyay AD, et al. Estimation of tau and phosphorylated Tau181 in serum of Alzheimer's disease and mild cognitive impairment patients. *PLoS One.* 2016;11(7):e0159099.
100. Moscoso A, Grothe MJ, Ashton NJ, Karikari TK, Rodríguez JL, Snellman A, et al. Time course of phosphorylated-tau181 in blood across the Alzheimer's disease spectrum. *Brain.* 2021;144(1):325–39.
101. Zettergren A, Lord J, Ashton NJ, Benedet AL, Karikari TK, Lantero Rodríguez J, et al. Association between polygenic risk score of Alzheimer's disease and plasma phosphorylated tau in individuals from the Alzheimer's disease neuroimaging initiative. *Alzheimers Res Ther.* 2021;13(1):17.
102. Simrén J, Leuz A, Karikari TK, Hye A, Benedet AL, Lantero Rodríguez J, et al. The diagnostic and prognostic capabilities of plasma biomarkers in Alzheimer's disease. *Alzheimers Dement.* 2021. <https://doi.org/10.1002/alz.12583>.
103. Keshavan A, Pannet J, Karikari TK, Rodríguez JL, Ashton NJ, Nicholas JM, et al. Population-based blood screening for preclinical Alzheimer's disease in a British birth cohort at age 70. *Brain.* 2021;awaa403. <https://doi.org/10.1093/brain/awaa403>.
104. Palmqvist S, Janelidze S, Quiroz YT, Zetterberg H, Lopera F, Stomrud E, et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. *JAMA.* 2020;324(8):772–81.

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APPENDIX C: Ding et al., Scientific Commentaries. Brain (2020)

SCIENTIFIC COMMENTARIES

Plasma p-tau217: from ‘new kid’ to most promising candidate for Alzheimer’s disease blood test

This scientific commentary refers to ‘Longitudinal plasma p-tau217 is increased in early stages of Alzheimer’s disease’, by Mattsson-Carlgrén *et al.* (doi:10.1093/brain/awaa286).

Alzheimer’s disease is characterized by a long presymptomatic phase during which neuropathology gradually accumulates. Detecting this pathology at the earliest possible stage offers the best chance of effective treatment. Clinical trials targeting the key pathological hallmarks of Alzheimer’s disease, such as cerebral amyloid- β aggregation and tangle formation, therefore focus on enrolling non-demented individuals with early-stage biomarker-defined disease. A reliable blood test for Alzheimer’s disease would have major implications for the selection of clinical trial participants by allowing non-demented individuals to be pre-screened in a minimally invasive and cost-effective manner, reducing the numbers who must undergo further testing with more invasive and expensive measures such as CSF analyses or PET scans.

Studies analysing plasma amyloid have shown that pre-screening with a blood test can significantly reduce further testing with more invasive measures (Verberk *et al.*, 2018), while more recent evidence suggests that measures of plasma phosphorylated tau (p-tau) may have greater diagnostic power than plasma amyloid measures

(Janelidze *et al.*, 2020; Thijssen *et al.*, 2020). Cross-sectional studies covering the complete clinical Alzheimer’s disease continuum have shown that plasma isoforms p-tau181 and p-tau217 can differentiate amyloid-PET or tau-PET positive cases from amyloid-PET or tau-PET negative cases (Janelidze *et al.*, 2020; Karikari *et al.*, 2020; Palmqvist *et al.*, 2020; Thijssen *et al.*, 2020). These cross-sectional studies have also shown that plasma p-tau measures can distinguish patients with Alzheimer’s disease dementia from those with frontotemporal lobar degeneration (Janelidze *et al.*, 2020; Karikari *et al.*, 2020; Palmqvist *et al.*, 2020; Thijssen *et al.*, 2020). In this issue of *Brain*, a timely longitudinal study by Mattsson-Carlgrén and co-workers extends this work to the preclinical stages of Alzheimer’s disease, and shows the value of using p-tau217 for participant selection in clinical trials as well as for disease monitoring (Mattsson-Carlgrén *et al.*, 2020).

Mattsson-Carlgrén *et al.* included 250 non-demented individuals from the Swedish BioFINDER study, and measured p-tau217 levels at baseline and during follow-up using the ‘Meso Scale Discovery’ (MSD) Eli Lilly immunoassay. The results showed that p-tau217 was increased in individuals in the preclinical and early clinical stages of Alzheimer’s disease when compared to cognitively healthy controls. In addition, higher p-tau217

levels were associated with a greater risk of converting to Alzheimer’s disease dementia and with steeper rates of cognitive decline and thinning of the temporal cortex and hippocampus. As well as showing that p-tau217 could be used to identify participants for inclusion in clinical trials, the results also suggest that p-tau217 could be used to monitor treatment responses over time. The authors showed that p-tau217 levels increased more steeply in non-demented individuals with Alzheimer’s disease pathology (amyloid positivity) than in those with no evidence of such pathology (Fig. 1). p-Tau217 levels also rose more steeply in non-demented individuals who developed Alzheimer’s disease dementia compared to those who remained non-demented during follow-up (Fig. 1). The logical next step would therefore be to analyse p-tau217 in blood samples obtained during trials that show reduction or even complete clearance of amyloid in the brain (Sevigny *et al.*, 2016) to determine whether p-tau217 could help monitor treatment response.

Results to date suggest that p-tau217 could also play a major role in a clinical diagnostic setting. For a biomarker to be used in the clinic, three phases of development must be completed (Fig. 2). The first phase, which focuses on assay development and validation plus initial clinical validation, has almost been completed for p-tau217. The Eli Lilly assay has shown robust

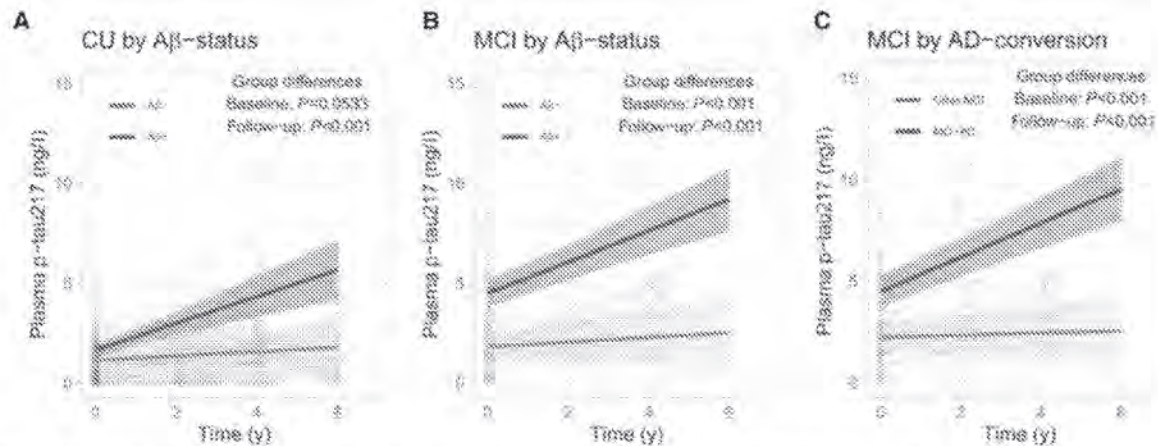


Figure 1 Longitudinal plasma p-tau217 data from Mattsson-Carligen *et al.* (2020). Subject-specific biomarker data are shown together with main effects from linear mixed effects models, adjusted for age and sex, for p-tau217 in (A) amyloid- β -negative ($A\beta^-$) cognitively unimpaired (CU) versus amyloid- β -positive ($A\beta^+$) cognitively unimpaired; (B) amyloid- β -negative mild cognitive impairment (MCI) versus amyloid- β -positive MCI; and (C) MCI to Alzheimer's disease (AD) dementia converters versus the remaining MCI population (i.e. stable MCI or 'MCI to other dementia' converters).

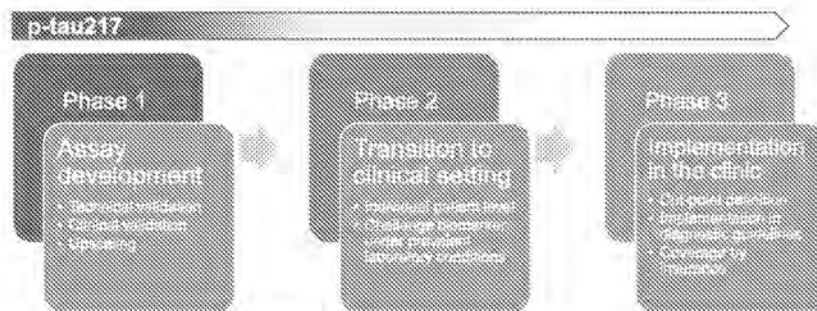


Figure 2 Three-phase trajectory of biomarker development.

outcomes (Palmqvist *et al.*, 2020), although the results of assay validation studies have yet to be published. The second phase focuses on the transition from a research setting to a clinical setting, with the aim of ensuring that the results are consistent at the level of individual patients in different settings and to define the context of use. Here, clinical evaluation should focus on establishing large databases containing information on the plasma p-tau217 concentrations of individual patients alongside their clinical and biomarker characteristics. During this phase, work on the assay includes assessing its robustness, developing reference material, and testing biomarker stability under prevalent

conditions. This may include testing the variation introduced by delays in processing samples or by the need to cool samples and transport them to the laboratory. The third phase focuses on the final definition of cut-points based on the data obtained in phase 2, and on evaluation of the assay results in real-life clinical settings in unselected patients. In parallel, insurance coverage of plasma testing for diagnostic purposes needs to be arranged where required, and the biomarker positioned in clinical diagnostic guidelines. Currently, we are close to reaching the end of phase one for p-tau217. It is thus time to transition to phases two and three, aiming for a swift implementation of

the plasma p-tau217 biomarker in both trials and clinical practice, to accelerate drug development and improve clinical care for Alzheimer's disease.

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doi:10.1093/brain/awaa329

Competing interests

The authors report no competing interests.

References

- Janelidze S, Mattsson N, Palmqvist S, Smith R, Beach TG, Serrano GE, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat Med* 2020; 26: 379–86.
- Karikari TK, Pascoal TA, Ashton NJ, Janelidze S, Beneder AL, Rodriguez JL, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol* 2020; 19: 422–33.
- Mattsson-Carlgen N, Janelidze S, Palmqvist S, Cullen N, Svenningsson AL, Strandberg O, et al. Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease. *Brain* 2020; 143: 3234–41.
- Palmqvist S, Janelidze S, Quiroz YT, Zetterberg H, Lopera F, Stomrud E, et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. *JAMA* 2020; 324: 772–81.
- Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M, et al. The antibody aducanumab reduces A β plaques in Alzheimer's disease. *Nature* 2016; 537: 50–6.
- Thijssen EH, La Joie R, Wolf A, Strom A, Wang P, Iaccarino L, et al. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. *Nat Med* 2020; 26: 387–97.
- Verberk IMW, Slot RE, Verfaillie SCJ, Heijst H, Prins ND, van Berckel BNM, et al. Plasma amyloid as prescreener for the earliest Alzheimer pathological changes. *Ann Neurol* 2018; 84: 648–58.

Is bad brain worse than no brain? Salvaging the cerebral cortex in epilepsy

This scientific commentary refers to 'Resective surgery prevents progressive cortical thinning in temporal lobe epilepsy', by Galovic *et al.* (doi:10.1093/brain/awaa284).

The human cerebral cortex, whose thickness ranges from 1 to 4 mm depending on the region, has one of the highest neuronal densities of all species. This highly folded sheet of cells is responsible for many of the higher cognitive functions that characterize humans. Cortical thickness, a proxy for cortical structural integrity, is dynamic; it changes with growth, maturation and ageing. However, our increasing ability to assess the cortex *in vivo* has also revealed associations between cortical thickness and factors such as sex, genetic background, cognitive function and disease states. Cortical thinning that progresses at a rate higher than expected for age has been demonstrated in a number of neurological and psychiatric conditions, including schizophrenia, depression, dementia and neurodegenerative disorders, multiple sclerosis and, not least, epilepsy. In this issue of *Brain*, Galovic and co-workers shed new light on this important area of epilepsy research (Galovic *et al.*, 2020).

That loss of brain tissue occurs in chronic epilepsy has been known for many years, as a result of structural brain imaging and the analysis of pathological specimens. What has been less well studied until more recently is the relative contribution of cortical thinning to overall brain tissue loss in epilepsy. Other unanswered questions include the histopathological and pathophysiological underpinnings of cortical thinning, its rate of progression and topography, its association with specific clinical aspects of epilepsy, and importantly, its response to effective epilepsy surgery.

One concept that is closely related to cortical thinning in epilepsy—a process that occurs over time—is that of epilepsy as a progressive disorder. Progressive structural brain changes in epilepsy include neuronal damage and loss, dendritic and synaptic reorganization, axonal loss and thinning, neurodegenerative changes, and gliosis and microgliosis. Progressive functional brain changes in epilepsy include the emergence of the abnormal connectivity and synaptic circuitry that sustains epileptogenesis and that gives rise to abnormal cognitive function. The severity of epilepsy can also increase over time, with the extent of the

increase depending on the type of epilepsy or epilepsy syndrome, and the patient's age and genetic background (Pitkänen and Sutula, 2002).

The notion that seizures themselves contribute to the observed progressive worsening of epilepsy and cognitive function is supported by observations from basic research (Pitkänen and Sutula, 2002). As early as the 19th century, Gowers proposed that 'seizures beget seizures', and in the 1950s, Penfield and Jasper introduced the concept of 'nociferous cortex' in patients with epilepsy (Penfield and Jasper, 1954). This term was applied to cerebral cortex that does not function normally, is the origin of epilepsy, impairs the function of other brain areas, and whose resection results in dramatic improvement of function, behaviour and seizures. Clinical observations, however, have yielded less consistent results with regard to the progressive nature of epilepsy and cognitive decline, and regarding the role of the epileptic process and the nociferous cortex.

Recent advances in quantitative MRI have produced objective imaging biomarkers of disease progression that allow us to measure the rate of age-adjusted cortical thinning in a variety of



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NOTICE OF ALLOWANCE AND FEE(S) DUE

23628 7590 11/02/2021
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

EXAMINER

WEIDNER, ADAM M

ART UNIT

PAPER NUMBER

1649

DATE MAILED: 11/02/2021

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/522,237	07/25/2019	David Wilson	Q0052.70018US03	4736

TITLE OF INVENTION: METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1200	\$0.00	\$0.00	\$1200	02/02/2022

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
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P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

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Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/522,237	07/25/2019	David Wilson	Q0052.70018US03	4736

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APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1200	\$0.00	\$0.00	\$1200	02/02/2022

EXAMINER	ART UNIT	CLASS-SUBCLASS
WEIDNER, ADAM M	1649	435-007920

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

- (1) The names of up to 3 registered patent attorneys or agents OR, alternatively, 1 _____
- (2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. 2 _____
- 3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. Fees submitted: ☐ Issue Fee ☐ Publication Fee (if required) ☐ Advance Order - # of Copies _____

4b. Method of Payment: (Please first reapply any previously paid fee shown above)

- ☐ Electronic Payment via EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)
- ☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. Change in Entity Status (from status indicated above)

- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
- ☐ Applicant asserting small entity status. See 37 CFR 1.27
- ☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____



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23628 7590 11/02/2021 WOLF GREENFIELD & SACKS, P.C. 600 ATLANTIC AVENUE BOSTON, MA 02210-2206			EXAMINER WEIDNER, ADAM M	
			ART UNIT	PAPER NUMBER
			1649	
DATE MAILED: 11/02/2021				

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.** Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 16/522,237	Applicant(s) Wilson et al.	
	Examiner ADAM M WEIDNER	Art Unit 1649	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to 7/16/21.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are 11,20-24 and 92-106. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

- a) ☐ All b) ☐ Some* c) ☐ None of the:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.

THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.
Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

- | | |
|--|--|
| 1. ☐ Notice of References Cited (PTO-892) | 5. ☐ Examiner's Amendment/Comment |
| 2. ☒ Information Disclosure Statements (PTO/SB/08),
Paper No./Mail Date _____ | 6. ☒ Examiner's Statement of Reasons for Allowance |
| 3. ☐ Examiner's Comment Regarding Requirement for Deposit
of Biological Material _____ | 7. ☐ Other _____ |
| 4. ☐ Interview Summary (PTO-413),
Paper No./Mail Date _____ | |

/Adam Weidner/
Primary Examiner, Art Unit 1649

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

Information Disclosure Statement

The 3-page information disclosure statement filed 7/16/21 fails to comply with the provisions of 37 CFR 1.97, 1.98 and MPEP § 609 because it fails to comply with the formal requirements of 1.98, such as failing to include a column for the Examiner's initials. It has been placed in the application file, but the information referred to therein has not been considered as to the merits. Applicant is advised that the date of any re-submission of any item of information contained in this information disclosure statement or the submission of any missing element(s) will be the date of submission for purposes of determining compliance with the requirements based on the time of filing the statement, including all certification requirements for statements under 37 CFR 1.97(e). See MPEP § 609.05(a).

Reasons for Allowance

The following is an examiner's statement of reasons for allowance:

The Examiner established a *prima facie* case of obviousness (see previous action). Once this has been done, burden shifts to Applicant to present evidence to rebut this *prima facie* case (MPEP §2145). In this case, Applicant presents Ding, presented as appendix B and attached to the interview summary mailed 8/2/21; this document is also cited on the IDS filed 7/16/21. Of particular relevance, Ding table 1 lists Applicant's claimed assay (tau Simoa) used by at least forty-two (42) groups after the effective filing date of 4/12/11. This is in contrast to the obviousness conclusion set forth by Examiner, which articulated why it would have been obvious to use Simoa to detect tau, but also recognizing that no group had actually done so.

As per MPEP §2145, rebuttal evidence may be in the form of establishing commercial success, including "evidence that the claimed invention was copied by others. See, e.g., *In re GPAC*, 57 F.3d 1573, 1580, 35 USPQ2d 1116, 1121 (Fed. Cir. 1995); *Hybritech Inc.v. Monoclonal Antibodies*, 802 F.2d 1367, 1380, 231 USPQ 81, 90 (Fed. Cir. 1986)".

In this case, no group prior to the effective filing date had done more than perhaps suggest using Simoa to detect tau (see Quanterix document previously discussed), while dozens of groups utilized Simoa to detect tau after the date of Applicant's contribution. It is on this fact that the Examiner finds the argument persuasive and concludes that the rebuttal evidence is sufficient to overcome the obviousness rejection.

A terminal disclaimer was filed and accepted, obviating the double patenting rejection.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ADAM M WEIDNER whose telephone number is (571)272-3045. The examiner can normally be reached M-F 9-16.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Stucker can be reached on 571-272-0911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: <https://patentcenter.uspto.gov>. Visit <https://www.uspto.gov/patents/apply/patent-center> for more information about Patent Center and <https://www.uspto.gov/patents/docx> for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance

Application/Control Number: 16/522,237
Art Unit: 1649

Page 4

from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Adam Weidner/
Primary Examiner, Art Unit 1649

Issue Classification 	Application/Control No. 16/522,237	Applicant(s)/Patent Under Reexamination Wilson et al.
	Examiner ADAM M WEIDNER	Art Unit 1649

CPC						
Symbol					Type	Version
G01N	/	33	/	6896	F	2013-01-01
G01N	/	33	/	54306	I	2013-01-01
G01N	/	2800	/	28	A	2013-01-01
G01N	/	2333	/	4709	A	2013-01-01
G01N	/	2333	/	47	A	2013-01-01
G01N	/	2800	/	52	A	2013-01-01

CPC Combination Sets					
Symbol	Type	Set	Ranking	Version	
/		/			

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	21	
/Adam Weidner/ Primary Examiner, Art Unit 1649	23 October 2021	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

U.S. Patent and Trademark Office

Part of Paper No.: 20211023

Issue Classification 	Application/Control No. 16/522,237	Applicant(s)/Patent Under Reexamination Wilson et al.
	Examiner ADAM M WEIDNER	Art Unit 1649

INTERNATIONAL CLASSIFICATION			
CLAIMED			
G01N33/68	/	33	/ 68
G01N33/543	/	33	/ 543
NON-CLAIMED			
	/		/

US ORIGINAL CLASSIFICATION	
CLASS	SUBCLASS

CROSS REFERENCES(S)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)					

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	21	
/Adam Weidner/ Primary Examiner, Art Unit 1649	23 October 2021	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

U.S. Patent and Trademark Office

Part of Paper No.: 20211023

Issue Classification 	Application/Control No. 16/522,237	Applicant(s)/Patent Under Reexamination Wilson et al.
	Examiner ADAM M WEIDNER	Art Unit 1649

☐ Claims renumbered in the same order as presented by applicant
 ☐ CPA
 ☒ T.D.
 ☐ R.1,47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	92	10	101	19	22										
2	93	11	102	20	23										
3	94	12	105	21	24										
4	95	13	106												
5	96	14	10												
6	97	15	104												
7	98	16	103												
8	99	17	11												
9	100	18	21												

NONE (Assistant Examiner) _____ (Date) _____		Total Claims Allowed: 21	
/Adam Weidner/ Primary Examiner, Art Unit 1649 (Primary Examiner) _____ (Date) _____		23 October 2021 (Date)	O.G. Print Claim(s) 1
			O.G. Print Figure 1

U.S. Patent and Trademark Office

Part of Paper No.: 20211023

Search Notes 	Application/Control No. 16/522,237	Applicant(s)/Patent Under Reexamination Wilson et al.
	Examiner ADAM M WEIDNER	Art Unit 1649

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
inventor/assignee east	03/11/2021	amw
review prosecution of parent 15/269142 and 14/111326	03/11/2021	amw
inventor/assignee east/palm	10/23/2021	amw

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
	see east notes	10/23/2021	amw

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DOCKET NO.: Q0052.70018U803

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: David Wilson
Application No.: 16/522,237
Confirmation No.: 4736
Filed: July 25, 2019
For: METHODS OF DETERMINING A TREATMENT PROTOCOL FOR
AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A
BRAIN INJURY
Examiner: A. M. Weidner
Art Unit: 1649

Certificate of Electronic Filing under 37 C.F.R. § 1.8
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(b)(4).

Dated: July 16, 2021

Electronic Signature for: /Eileen M. MacKenzie/

MAIL STOP AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**STATEMENT FILED PURSUANT TO THE DUTY OF
DISCLOSURE UNDER 37 C.F.R. §§ 1.56, 1.97 AND 1.98**

Sir:

Pursuant to the duty of disclosure under 37 C.F.R. §§ 1.56, 1.97 and 1.98, the undersigned requests consideration of this Information Disclosure Statement.

PART I: Compliance with 37 C.F.R. § 1.97

This Information Disclosure Statement has been filed more than three months after the filing date of this application and after the mailing date of a first Office Action, but before the mailing date of any of a Final Action under 37 C.F.R. § 1.113, a Notice of Allowance under 37 C.F.R. § 1.311, or an action that otherwise closes prosecution in this application.

Please charge our Credit Card in the amount of \$260.00 covering the fee set forth in 37 C.F.R. § 1.17(p).

9410383.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /AMW/

Application No.: 16/522,237
Conf. No.: 4736

- 2 -

Art Unit: 1649

PART II: Information Cited

The undersigned hereby makes of record in the above-identified application the information listed on the attached form PTO-1449 (modified PTO/SB/08). The order of presentation of the references should not be construed as an indication of the importance of the references.

The undersigned hereby makes the following additional information of record in the above-identified application.

The undersigned would like to bring to the Examiner's attention the following co-pending applications that may contain subject matter related to this application:

<u>Serial No.</u>	<u>Filing Date</u>	<u>Inventor(s)</u>	<u>Docket No.</u>
*17/210,258	03-23-2021	Rissin et al.	Q0052.70015US03

*A copy of this reference is not provided as the Office has waived the requirement under 37 C.F.R. § 1.98(a)(2)(iii) for submitting a copy of a cited U.S. patent application if it is scanned to the Image File Wrapper system and is available on Private PAIR.

PART III: Remarks

Documents cited anywhere in the Information Disclosure Statement are enclosed unless otherwise indicated. It is respectfully requested that:

1. The Examiner consider completely the cited information, along with any other information, in reaching a determination concerning the patentability of the present claims;
2. The enclosed form PTO-1449 (modified PTO/SB/08) be signed by the Examiner to evidence that the cited information has been fully considered by the United States Patent and Trademark Office during the examination of this application;
3. The citations for the information be printed on any patent which issues from this application.

By submitting this Information Disclosure Statement, the undersigned makes no representation that a search has been performed, of the extent of any search performed, or that more relevant information does not exist.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /AMW/

Application No.: 16/522.237
Conf. No.: 4736

- 3 -

Art Unit: 1649

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

By submitting this Information Disclosure Statement, the undersigned makes no representation that the information cited in the Statement is, or is considered to be, in fact, prior art as defined by 35 U.S.C. § 102, or an admission by Applicant as prior art.

Notwithstanding any statements by the undersigned, the Examiner is urged to form his or her own conclusion regarding the relevance of the cited information.

An early and favorable action is hereby requested.

The Director is hereby authorized to charge any deficiency or credit any overpayment in the fees occasioned by the filing of this Information Disclosure Statement to our Deposit Account No. 23/2825 under Docket No. Q0052.70018US03 from which the undersigned is authorized to draw.

Respectfully submitted,

Electronic signature: /Michael J. Pomianek/
Michael J. Pomianek
Registration No.: 46,190
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Docket No.: Q0052.70018US03
Date: July 16, 2021

/Adam Weidner/

10/23/2021

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /AMW/

Bibliographic Data

Application No: 16/522,237

Foreign Priority claimed: ☐ Yes ☒ No

35 USC 119 (a-d) conditions met: ☐ Yes ☐ No ☐ Met After Allowance

Verified and Acknowledged:

/Adam Weidner/

Examiner's Signature

Initials

Title:

METHODS OF DETERMINING A TREATMENT PROTOCOL FOR
AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A
BRAIN INJURY

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
07/25/2019	435	1649	Q0052.70018US03
RULE			

APPLICANTS

Quanterix Corporation, Billerica, MA, UNITED STATES

INVENTORS

David Wilson, Boxborough, MA, UNITED STATES

John Henrik Zetterberg, Molnlycke, SWEDEN

Kaj Blennow, Kungsbacka, SWEDEN

Jeffrey D. Randall, Canton, MA, UNITED STATES

CONTINUING DATA

This application is a CON of 15269142 09/19/2016 PAT 10393759

15269142 is a CON of 14111326 06/24/2014ABN

14111326 is a 371 of PCT/US2012/033343 04/12/2012

PCT/US2012/033343 has PRO of 61524693 08/17/2011

PCT/US2012/033343 has PRO of 61474315 04/12/2011

FOREIGN APPLICATIONS

IF REQUIRED, FOREIGN LICENSE GRANTED**

08/07/2019

STATE OR COUNTRY

UNITED STATES

ADDRESS

WOLF GREENFIELD & SACKS, P.C.

600 ATLANTIC AVENUE

BOSTON, MA 02210-2206

UNITED STATES

FILING FEE RECEIVED

\$3,280

FORM PTO-1449/A and B (modified PTO/SB/08) INFORMATION DISCLOSURE STATEMENT BY APPLICANT				APPLICATION NO.: 16/522,237		ATTY. DOCKET NO.: Q0052.70018US03	
				FILING DATE: July 25, 2019		CONFIRMATION NO.: 4736	
				FIRST NAMED INVENTOR: David Wilson			
				GROUP ART UNIT: 1649		EXAMINER: A. M. Weidner	
Sheet	1	of	1				

U.S. PATENT DOCUMENTS

Examiner's Initials *	Cite No.	U.S. Patent Document		Name of Patentee or Applicant of Cited Document	Date of Publication or Issue of Cited Document MM-DD-YYYY
		Number	Kind Code		
		10,989,713	B2	Rissin et al.	04-27-2021
		2020-0123592	A1	Diaz-Mochon et al.	04-23-2020

FOREIGN PATENT DOCUMENTS

Examiner's Initials *	Cite No.	Foreign Patent Document			Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY	Translation (Y/N)
		Office/Country	Number	Kind Code			

OTHER ART - NON PATENT LITERATURE DOCUMENTS

Examiner's Initials *	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	Translation (Y/N)
		CAIRNS, A new market beckons for Alzheimer's blood tests. Evaluate Vantage. 2021 Apr 19. https://www.evaluate.com/vantage/articles/analysis/spotlight/new-market-beckons-alzheimers-blood-tests [last accessed 15 July 2021]. 3 pages.	
		DING et al., Ultrasensitive assays for detection of plasma tau and phosphorylated tau 181 in Alzheimer's disease: a systematic review and meta-analysis. Transl Neurodegener. 2021 Mar 12;10(1):10.	
		LILIANG et al., Tau proteins in serum predict outcome after severe traumatic brain injury. J Surg Res. 2010 May 15;160(2):302-7.	
		OST et al., Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury. Neurology. 2006 Nov 14;67(9):1600-4.	
		TEUNISSEN et al., Plasma p-tau217: from 'new kid' to most promising candidate for Alzheimer's disease blood test. Brain. 2020 Dec 5;143(11):3170-3172.	

[NOTE - No copies of U.S. patents, published U.S. patent applications, or pending, unpublished patent applications stored in the USPTO's Image File Wrapper (IFW) system, are included. See 37 CFR § 1.98 and 1287OG163. Copies of all other patent(s), publication(s), unpublished, pending U.S. patent applications, or other information listed are provided as required by 37 CFR § 1.98 unless 1) such copies were provided in an IDS in an earlier application that complies with 37 CFR § 1.98, and 2) the earlier application is relied upon for an earlier filing date under 35 U.S.C. § 120.]

EXAMINER: /Adam Weidner/	DATE CONSIDERED: 10/23/2021
---------------------------------	------------------------------------

* EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to Applicant.

9410382.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /AMW/

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1002
000671

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L4	15	L3 and tau.clm.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2021/10/23 09:45
L3	8,515	L1 or L2	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2021/10/23 09:45
L2	8,474	((wilson near2 david) or (john near2 zetterber) or (kaj near2 blennow) or (jeffrey near2 randall)).in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2021/10/23 09:45
L1	56	quanterix.as.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO	ADJ	ON	2021/10/23 09:45

EAST Search History (Interference)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L6	21	L5 and hours.clm. and tau.clm. and blood.clm.	US-PGPUB; USPAT	ADJ	ON	2021/10/23 09:45
L5	16,981	(g01n33/6896 or g01n28/47 or g01n2333/47 or g01n2800/52).cpc.	US-PGPUB; USPAT	ADJ	ON	2021/10/23 09:45

10/23/2021 9:46:15 AM

C:\Users\aweidner\Documents\EAST\Workspaces\16522237.wsp

UNITED STATES PATENT AND TRADEMARK OFFICE
COMMISSIONER FOR PATENTS
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ALEXANDRIA VA 22313-1451

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WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206



**Courtesy Reminder for
Application Serial No: 16/522,237**

Attorney Docket No: Q0052.70018US03
Customer Number: 23628
Date of Electronic Notification: 11/02/2021

This is a courtesy reminder that new correspondence is available for this application. If you have not done so already, please review the correspondence. The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

An email notification regarding the correspondence was sent to the following email address(es) associated with your customer number:

Patents_eOfficeAction@WolfGreenfield.com
WGS_eOfficeAction@WolfGreenfield.com

To view your correspondence online or update your email addresses, please visit us anytime at **<https://ppair-my.uspto.gov/pair/PrivatePair>**.

If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov or call 1-866-217-9197.

Docket No.: Q0052.70018US03
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: David Wilson
Application No.: 16/522,237
Confirmation No.: 4736
Filed: July 25, 2019
For: METHODS OF DETERMINING A TREATMENT PROTOCOL FOR
AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A
BRAIN INJURY
Examiner: A. M. Weidner
Art Unit: 1649

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: February 1, 2022

Signature for Lucie DeBellis: /Lucie DeBellis/

AMENDMENT AFTER ALLOWANCE UNDER 37 C.F.R. § 1.312

Mail Stop Issue Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INTRODUCTORY COMMENTS

Prior to issuance of the patent, applicant respectfully requests entry of this amendment under 37 C.F.R. § 1.312.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

AMENDMENTS TO THE CLAIMS

1. - 10. (Canceled)

11. (Previously Presented) The method of claim 104, wherein the brain injury results from a hypoxic event.

12. - 19. (Canceled)

20. (Previously Presented) The method claim 92, wherein the limit of detection for the analytical protein concentration measurement assay is less than about 0.02 pg/mL.

21. (Previously Presented) The method of claim 104, wherein the brain injury comprises a hypoxic event caused by cardiac arrest.

22. (Previously Presented) The method of claim 104, wherein the brain injury comprises a hypoxic event caused by stroke.

23. (Previously Presented) The method of claim 104, wherein the brain injury comprises a hypoxic event caused by an ischemic event.

24. (Previously Presented) The method of claim 104, wherein the brain injury comprises a hypoxic event caused by a thrombosis.

25. - 91. (Cancelled)

92. (Previously Presented) A method of producing a bodily fluid sample of a patient suspected of having a neurological condition containing an analytically quantified amount of endogenous tau protein, said method comprising:

(A) obtaining a volume of bodily fluid comprising blood, or a blood component selected from plasma and serum,

(B) diluting the volume of bodily fluid, and

(C) quantifying through the use of an analytical protein concentration measurement assay a concentration of tau protein in the volume of bodily fluid to produce the bodily fluid sample containing the analytically quantified amount of endogenous tau protein;

wherein the analytically quantified concentration of endogenous tau protein contained in the bodily fluid sample is less than about 5 pg/ml, and a limit of quantification of the analytical protein concentration measurement assay used in step (C) for quantifying the concentration of tau protein is less than about 0.2 pg/mL.

93. (Previously Presented) The method of claim 92, wherein the analytical protein concentration measurement assay used in step (C) comprises:

i. exposing a plurality of capture objects that each include a binding surface having affinity for tau protein molecules to the volume of bodily fluid;

ii. immobilizing at least a portion of the tau protein molecules with respect to the plurality of capture objects such that at least some of the capture objects associate with at least one tau protein molecule and a statistically significant fraction of the capture objects do not associate with any tau protein molecules;

iii. determining a proportion of the plurality of capture objects that associated with a tau protein molecule in step ii; and

iv. determining a measure of tau protein molecule concentration in the volume of bodily fluid based at least in part on the proportion determined in step iii.

94. (Previously Presented) The method of claim 93, wherein the plurality of capture objects comprises a plurality of beads.

95. (Currently Amended) The method of claim 92, wherein the analytical protein concentration measurement assay used in step (C) comprises spatially segregating tau protein molecules into a

plurality of locations, wherein a statistically significant fraction of the locations contain a single tau protein molecule and a statistically significant fraction of the locations do not contain any tau protein molecules.

96. (Previously Presented) The method of claim 95, wherein the plurality of locations comprises a plurality of reaction vessels.

97. (Previously Presented) The method of claim 92, wherein each of the tau protein molecules quantified in step (C) is selected from the group consisting of tau 23 (352, 0N3R), tau 24 (383, 0N4R), tau 37 (381, 1N3R), tau 34 (412, 1N4R), tau 39 (410, 2N3R), and tau 40 (441, 2N4R).

98. (Previously Presented) The method of claim 97, wherein at least a portion of the tau protein molecules are phosphorylated.

99. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid comprises blood.

100. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid comprises plasma.

101. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid comprises serum.

102. (Previously Presented) The method of claim 92, wherein the volume of bodily fluid does not comprise cerebrospinal fluid (CSF).

103. (Previously Presented) The method of claim 104, wherein the volume of bodily fluid has been collected from the patient within 12 hours of the brain injury.

104. (Previously Presented) The method of claim 92, wherein the patient suspected of having a neurological condition is recovering from a brain injury.

105. (Previously Presented) The method of claim 92, wherein the analytical protein concentration measurement assay used in step (C) comprises:

- i. suspending a plurality of capture objects that each include a binding surface having affinity for tau protein molecules in the diluted volume of bodily fluid,
- ii. immobilizing at least a portion of the tau protein molecules with respect to at least some of the plurality of capture objects suspended in the diluted volume of bodily fluid such that at least some of the capture objects associate with at least one tau protein molecule from the volume of bodily fluid,
- iii. interrogating at least some of the capture objects associated with at least one tau molecule from the volume of bodily of fluid; and
- iv. determining a measure of tau protein molecule concentration in the volume of bodily fluid based at least in part on the interrogating step performed in step iii.

106. (Previously Presented) The method of claim 105, wherein the plurality of capture objects comprises a plurality of beads.

REMARKS

Claims 11, 20-24 and 92-106 were pending in the application. Claim 95 has been amended to add a period at the end of the claim. No new matter has been added. Claims 11, 20-24 and 92-106 are pending in this application with claim 92 being the sole independent claim.

If there is a fee occasioned by the amendment, including extension fee, the Director is hereby authorized to charge any deficiency or credit any overpayment in the fees filed, asserted to be filed or which should have been filed herewith to Deposit Account No. 23/2825, under Docket No. Q0052.70018US03 from which the undersigned is authorized to draw.

Dated: February 1, 2022

Respectfully submitted,

By: /Michael J. Pomianek/
Michael J. Pomianek
Registration No.: 46,190
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

Electronic Patent Application Fee Transmittal				
Application Number:		16522237		
Filing Date:		25-Jul-2019		
Title of Invention:		METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY		
First Named Inventor/Applicant Name:		David Wilson		
Filer:		Michael Pomianek/Lucie DeBellis		
Attorney Docket Number:		Q0052.70018US03		
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
UTILITY APPL ISSUE FEE	1501	1	1200	1200

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				1200

Electronic Acknowledgement Receipt	
EFS ID:	44885132
Application Number:	16522237
International Application Number:	
Confirmation Number:	4736
Title of Invention:	METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY
First Named Inventor/Applicant Name:	David Wilson
Customer Number:	23628
Filer:	Michael Pomianek/Lucie DeBellis
Filer Authorized By:	Michael Pomianek
Attorney Docket Number:	Q0052.70018U503
Receipt Date:	01-FEB-2022
Filing Date:	25-JUL-2019
Time Stamp:	06:21:51
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	\$1200
RAM confirmation Number	E202221722140095
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	Q005270018US03-TRN-MJP.pdf	26379	no	1
			2112811f5c0362a0f2631e425f75b6ac08f116f		
Warnings:					
Information:					
2	Issue Fee Payment (PTO-85B)	Q005270018US03-ISSFEE-MJP.pdf	38595	no	1
			1d7c8051eff7b661c09e6a6c06d91f4e21767e		
Warnings:					
Information:					
3	Post Allowance Communication- Incoming	Q005270018US03-CSRA-MJP.pdf	28122	no	2
			500df694e005704e701947d34e551a0819f1d9521		
Warnings:					
Information:					
4		Q005270018US03-312AMN-MJP.pdf	37825	yes	6
			00b0306d664d0e6c5d8ab97ace0b45d5d4a7721		
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Amendment after Notice of Allowance (Rule 312)		1	1	
	Claims		2	5	
	Applicant Arguments/Remarks Made in an Amendment		6	6	
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	38446	no	2
			009a434ae980602c16efb08c1b06c4570a7186c		

Warnings:	
Information:	
Total Files Size (in bytes):	169367
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.	

TRANSMITTAL FORM <i>(to be used for all correspondence after initial filing)</i>	Application Number	16/522,237-Conf. #4736
	Filing Date	July 25, 2019
	First Named Inventor	David Wilson
	Art Unit	1649
	Examiner Name	A. M. Weidner
Total Number of Pages in This Submission	Attorney Docket Number	Q0052.70018US03

ENCLOSURES (Check all that apply)		
☐ Fee Transmittal Form ☐ Fee Attached ☐ Amendment/Reply ☐ After Final ☐ Affidavits/declaration(s) ☐ Extension of Time Request ☐ Express Abandonment Request ☐ Information Disclosure Statement ☐ Certified Copy of Priority Document(s) ☐ Reply to Missing Parts/Incomplete Application ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53	☐ Drawing(s) ☐ Licensing-related Papers ☐ Petition ☐ Petition to Convert to a Provisional Application ☐ Power of Attorney, Revocation Change of Correspondence Address ☐ Terminal Disclaimer ☐ Request for Refund ☐ CD, Number of CD(s) _____ ☐ Landscape Table on CD	☐ After Allowance Communication to TC ☐ Appeal Communication to Board of Appeals and Interferences ☐ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) ☐ Proprietary Information ☐ Status Letter ☒ Other Enclosure(s) (please identify below): • Part B - Fee Transmittal • Comments on Statement of Reasons for Allowance • Amendment After Allowance
Remarks		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT			
Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Michael J. Pomianek/		
Printed name	Michael J. Pomianek		
Date	February 1, 2022	Reg. No.	46,190

Certificate of Electronic Filing under 37 C.F.R. § 1.8	
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).	
Dated: February 1, 2022	Signature for Lucie DeBellis: /Lucie DeBellis/

PART B—FEE(S) TRANSMITTAL

Complete and send this form, together with the applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

By fax, send to: (571) 273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first-class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

Lucie DeBellis	(Typed or printed name)
/Lucie DeBellis/	(Signature)
February 1, 2022	(Date)

APPLICATION NO	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO	CONFIRMATION NO
16/522,237	07/25/2019	David Wilson	Q0052.70018US03	4736

TITLE OF INVENTION: METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1,200.00			\$1,200.00	02/02/2022

EXAMINER	ART UNIT	CLASS-SUBCLASS
A. M. Weidner	1649	

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363)

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

- (1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1. Wolf, Greenfield & Sacks, P.C.

2.

3.

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE or COUNTRY)

Quanterix Corporation

Billerica, Massachusetts

Please check the appropriate assignee category or categories (will not be printed on the patent):

☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. Fees Submitted: ☒ Issue Fee ☐ Publication Fee (if required) ☐ Advance Order. # of Copies _____

4b. Method of Payment (Please first reuply any previously paid fee shown above):

☒ Electronic Payment via EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)

☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. 23/2825

5. Change of Entity Status (from status indicated above)

- ☐ Applicant certifying micro entity status. See 37 CFR 1.29.
☐ Applicant asserting small entity status. See 37 CFR 1.27.
☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid Certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken as a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken as a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Michael J. Pomianek/

Date February 1, 2022

Typed or printed name Michael J. Pomianek

Registration No. 46,190

9935906.1

Docket No.: Q0052.70018US03
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: David Wilson
Application No.: 16/522,237
Confirmation No.: 4736
Filed: July 25, 2019
For: METHODS OF DETERMINING A TREATMENT
PROTOCOL FOR AND/OR A PROGNOSIS OF A
PATIENT'S RECOVERY FROM A BRAIN INJURY
Examiner: A. M. Weidner
Art Unit: 1649

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: February 1, 2022

Signature for Lucie DeBellis: /Lucie DeBellis/

**COMMENTS ON STATEMENT OF REASONS
FOR ALLOWANCE UNDER 37 C.F.R. § 1.104(e)**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Applicant received the Examiner's Statement of Reasons for Allowance with the November 2, 2021 Notice of Allowance and Allowability for this application. Entry of the Statement into the record should not be construed as agreement with or acquiescence in the reasoning stated by the Examiner. Each claim stands on its own merits and is patentable because of the combination it recites and not because of the presence or absence of any one particular element.

The Examiner's Statement was not prepared by Applicant and only contains the Examiner's personal reasons for allowability. Thus, any interpretation with respect to the Examiner's Statement of Reasons for Allowance should not be imputed to the Applicant.

Applicant believes no fee is due with this Comments on Statement of Reasons for Allowance (37 CFR 1.104(e)). However, if a fee is due with this Comments on Statement of

Application No.: 16/522,237

2

Docket No.: Q0052.70018US03

Reasons for Allowance (37 CFR 1.104(e)), please charge our Deposit Account No. 23/2825, under Docket No. Q0052.70018US03 from which the undersigned is authorized to draw.

Dated: February 1, 2022

Respectfully submitted,

By: /Michael J. Pomianek/
Michael J. Pomianek
Registration No.: 46,190
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

9935902.1



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/522,237	07/25/2019	David Wilson	Q0052.70018US03	4736
23628	7590	02/15/2022	EXAMINER	
WOLF GREENFIELD & SACKS, P.C. 600 ATLANTIC AVENUE BOSTON, MA 02210-2206			WEIDNER, ADAM M	
			ART UNIT	PAPER NUMBER
			1649	
			NOTIFICATION DATE	DELIVERY MODE
			02/15/2022	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Patents_eOfficeAction@WolfGreenfield.com
WGS_eOfficeAction@WolfGreenfield.com

Response to Rule 312 Communication	Application No.	Applicant(s)	
	16/522,237	Wilson et al.	
	Examiner	Art Unit	AIA (FITF) Status
	ADAM M WEIDNER	1649	No

– The MAILING DATE of this communication appears on the cover sheet with the correspondence address –

1. ☒ The amendment filed on 01 February 2022 under 37 CFR 1.312 has been considered, and has been:

a) ☐ entered.

b) ☒ entered as directed to matters of form not affecting the scope of the invention.

c) ☐ disapproved because the amendment was filed after the payment of the issue fee.
Any amendment filed after the date the issue fee is paid must be accompanied by a petition under 37 CFR 1.313(c)(1) and the required fee to withdraw the application from issue.

d) ☐ disapproved. See explanation below.

e) ☐ entered in part. See explanation below.

/Adam Weidner/ Primary Examiner, Art Unit 1649	
---	--

OK TO ENTER: /AMW/

Docket No.: Q0052.70018US03
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: David Wilson
Application No.: 16/522,237
Confirmation No.: 4736
Filed: July 25, 2019
For: METHODS OF DETERMINING A TREATMENT PROTOCOL FOR
AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A
BRAIN INJURY
Examiner: A. M. Weidner
Art Unit: 1649

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: February 1, 2022

Signature for Lucie DeBellis: /Lucie DeBellis/

AMENDMENT AFTER ALLOWANCE UNDER 37 C.F.R. § 1.312

Mail Stop Issue Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

INTRODUCTORY COMMENTS

Prior to issuance of the patent, applicant respectfully requests entry of this amendment under 37 C.F.R. § 1.312.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 6 of this paper.

9976468.1



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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Alexandria, Virginia 22313-1450
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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/522,237	03/15/2022	11275092	Q0052.70018US03	4736

23628 7590 02/23/2022
WOLF GREENFIELD & SACKS, P.C.
600 ATLANTIC AVENUE
BOSTON, MA 02210-2206

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment is 66 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

INVENTOR(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional inventors):

David Wilson, Boxborough, MA;
John Henrik Zetterberg, Mohnlycke, SWEDEN;
Kaj Blennow, Kungsbacka, SWEDEN;
Jeffrey D. Randall, Canton, MA;

APPLICANT(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional applicants):

Quanterix Corporation, Billerica, MA;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

Docket No.: Q0052.70018US03
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor: David Wilson
Application No.: 16/522,237
Confirmation No.: 4736
Filed: July 25, 2019
Patent No.: 11,275,092
Issued: March 15, 2022
For: METHODS OF DETERMINING A TREATMENT PROTOCOL FOR
AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A
BRAIN INJURY
Examiner: A. M. Weidner
Art Unit: 1649

Certificate of Electronic Filing under 37 C.F.R. § 1.8

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: March 17, 2022

Electronic Signature for: /Trish McDonald/

REQUEST FOR CERTIFICATE OF CORRECTION PURSUANT TO 37 CFR 1.322

Attention: Certificate of Correction Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Upon reviewing the above-identified patent, Patentee noted typographical errors which should be corrected.

In the Specification:

At column 1, line 27, the word "HYDPXIC" should read --HYPOXIC--

In the Claims:

At column 37, claim 1, line 36, the words "5 pg/ml" should read --5 pg/ml"

10133613.1

At column 38, claim 6, line 10, the words "tau 2.4" should read --tau 24--

At column 38, claim 18, line 54, the words "15 wherein" should read --15, wherein--

At column 38, claim 21, line 60, the words "15 wherein" should read --15, wherein--

This request does not involve correction of Applicant errors. Accordingly, no fee is required.

Transmitted herewith is a proposed Certificate of Correction effecting such amendment. Patentee respectfully solicits the granting of the requested Certificate of Correction.

Applicant believes no fee is due with this Request for Certificate of Correction -- PTO Error -- no fee required (37 CFR 1.322). However, if a fee is due with this Request for Certificate of Correction -- PTO Error -- no fee required (37 CFR 1.322), please charge our Deposit Account No. 23/2825, under Docket No. Q0052.70018US03 from which the undersigned is authorized to draw.

Dated: March 17, 2022

Respectfully submitted,

By: /Michael J. Pomianek/
Michael J. Pomianek
Registration No.: 46,190
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206
617.646.8000

**UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION**

Page 1 of 1

PATENT NO. : 11,275,092
APPLICATION NO. : 16/522,237
ISSUE DATE : March 15, 2022
INVENTOR(S) : David Wilson et al.

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification:

At column 1, line 27, the word "HYPDXIC" should read --HYPOXIC--

In the Claims:

At column 37, claim 1, line 36, the words "5 pg/ml" should read --5 pg/ml"

At column 38, claim 6, line 10, the words "tau 2.4" should read --tau 24--

At column 38, claim 18, line 54, the words "15 wherein" should read --15, wherein--

At column 38, claim 21, line 60, the words "15 wherein" should read --15, wherein--

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):
Michael J. Pomianek
WOLF, GREENFIELD & SACKS, P.C.
600 Atlantic Avenue
Boston, Massachusetts 02210-2206

1

10133612.1

Electronic Acknowledgement Receipt	
EFS ID:	45257871
Application Number:	16522237
International Application Number:	
Confirmation Number:	4736
Title of Invention:	METHODS OF DETERMINING A TREATMENT PROTOCOL FOR AND/OR A PROGNOSIS OF A PATIENT'S RECOVERY FROM A BRAIN INJURY
First Named Inventor/Applicant Name:	David Wilson
Customer Number:	23628
Filer:	Michael Pomianek/Patricia McDonald
Filer Authorized By:	Michael Pomianek
Attorney Docket Number:	Q0052.70018US03
Receipt Date:	17-MAR-2022
Filing Date:	25-JUL-2019
Time Stamp:	22:31:18
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1)	Miscellaneous Incoming Letter	Q005270018US03-TRN-MJP.pdf	26209 r7205d6c5aef11bd197d01e9e0dc1c97baf29f10	no	1

Warnings:

Information:					
2	Request for Certificate of Correction	Q005270018US03-RCOC-MJP.pdf	27683 file:///d:/595e5b7a21920918cd52d18c6ef9286c6	no	2
Warnings:					
Information:					
3	Request for Certificate of Correction	Q005270018US03-COC-MJP.pdf	17073 file:///c:/311600dca31c29d01600f80a1d0a66f08d9	no	1
Warnings:					
Information:					
Total Files Size (in bytes):			70965		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					

TRANSMITTAL FORM <i>(to be used for all correspondence after initial filing)</i>	Application Number	Patent#: 11,275,092
	Filing Date	Issued: March 15, 2022
	First Named Inventor	David Wilson
	Art Unit	1649
	Examiner Name	A. M. Weidner
Total Number of Pages in This Submission	Attorney Docket Number	Q0052.70018US03

ENCLOSURES (Check all that apply)		
☐ Fee Transmittal Form ☐ Fee Attached ☐ Amendment/Reply ☐ After Final ☐ Affidavits/declaration(s) ☐ Extension of Time Request ☐ Express Abandonment Request ☐ Information Disclosure Statement ☐ Certified Copy of Priority Document(s) ☐ Reply to Missing Parts/Incomplete Application ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53	☐ Drawing(s) ☐ Licensing-related Papers ☐ Petition ☐ Petition to Convert to a Provisional Application ☐ Power of Attorney, Revocation Change of Correspondence Address ☐ Terminal Disclaimer ☐ Request for Refund ☐ CD, Number of CD(s) _____ ☐ Landscape Table on CD	☐ After Allowance Communication to TC ☐ Appeal Communication to Board of Appeals and Interferences ☐ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) ☐ Proprietary Information ☐ Status Letter ☒ Other Enclosure(s) (please identify below): - Request for Certificate of Correction - Certificate of Correction
Remarks		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT			
Firm Name	WOLF, GREENFIELD & SACKS, P.C.		
Signature	/Michael J. Pomianek/		
Printed name	Michael J. Pomianek		
Date	March 17, 2022	Reg. No.	46,190

Certificate of Electronic Filing under 37 C.F.R. § 1.8	
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office's electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).	
Dated: March 17, 2022	Electronic Signature for: /Trish McDonald/

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 11,275,092 B2
APPLICATION NO. : 16/522237
DATED : March 15, 2022
INVENTOR(S) : David Wilson et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

At Column 1, Line 27, the word "HYPDXIC" should read --HYPOXIC--

In the Claims

At Column 37, Claim 1, Line 36, the words "5 pg/nil" should read --5 pg/ml--

At Column 38, Claim 6, Line 10, the words "tau 2.4" should read --tau 24--

At Column 38, Claim 18, Line 54, the words "15 wherein" should read --15, wherein--

At Column 38, Claim 21, Line 60, the words "15 wherein" should read --15, wherein--

Signed and Sealed this
Nineteenth Day of April, 2022
Katherine Kelly Vidal

Katherine Kelly Vidal
Director of the United States Patent and Trademark Office