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Analysis of Cocaine Analytes in Human Hair II: Evaluation of Different Hair Color and Ethnicity Types

Final Report

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ABSTRACT

The mechanism(s) of permeability of hair to drugs are not fully understood. Research data suggest that hair color may affect cocaine's incorporation into and retention in the hair matrix. The possibility that differences in hair color may cause one individual to be more likely to test positive for a drug than another, despite both having ingested or having been exposed to the same amount of a drug, greatly concerns policymakers and forensic practitioners. The potential for such bias must be understood to ensure the correct interpretation of results and the appropriate use of hair testing. If it is shown that hair color influences drug permeability, the current drug-testing methods may need improvement to take these variations into account and remove any potential for bias and false-positive results. The goal of this study was to evaluate cocaine analytes in hair of different color (e.g., light, dark) and ethnic origin (e.g., Caucasian, African American) after the hair was subjected to surface contamination with cocaine and subsequent laboratory decontamination.

The *in vitro* surface contamination study design followed a previously published method by Stout and colleagues. Briefly, verified drug-free head hair samples (Caucasian light and dark hair; African-American (AA) hair; n=12 each) were collected under IRB protocol, contaminated with cocaine hydrochloride powder, shampooed daily for 8 weeks (56 days) with aliquots removed weekly for decontamination (two decontamination protocols: methanol and extensive phosphate buffer), and subjected to cocaine analyte testing by LC-MS/MS. Quantitative analytical procedures for the determination of cocaine, benzoylecgonine, cocaethylene, and norcocaine in hair were performed on an Agilent Technologies (Santa Clara, CA) 1200 Series LC system coupled to a 6410 triple quadrupole mass spectrometer, operated in positive ESI mode. For confirmation, two transitions were monitored and one ion ratio was determined, which

was acceptable if within 20% of the ratio of known calibration standards. The limits of quantitation were 25 pg/mg cocaine and 2.5 pg/mg benzoylecgonine, cocaethylene, and norcocaine. The upper limit of linearity was 55,000 pg/mg for cocaine and 1,000 pg/mg for all other analytes. Between-run imprecision was less than 3% for cocaine at 150 pg/mg and less than 8% at 15 pg/mg for all other analytes.

While our previous cocaine surface contamination studies were designed to provide an estimate of intra or within-individual variation, this study includes sufficient samples to determine differences between ethnic groups or hair color with statistical significance. These data suggest there is no apparent simple relationship between concentration and ethnicity by this *in vitro* cocaine surface contamination model. SEM morphology and hair-staining studies by methylene blue and rhodamine staining dyes could not predict hair drug concentration or positive calls following cocaine surface contamination. AA hair had significantly more cocaine present, but lower benzoylecgonine/cocaine ratios than Caucasian hair, and therefore, fewer actual positive calls than Caucasian (blond and dark hair types) by current administrative cutoff concentrations. While these results are statistically significant, the current study is limited to a small population. Additional results demonstrate that methanol laboratory hair decontamination is much less effective compared to extended phosphate buffer decontamination by this *in vitro* surface contamination model. Finally, this study demonstrates a lack of relationship between SEM morphology and hair color or ethnicity, as well as no relationship between dye staining of hair and cocaine concentration or positive results of the hair.

The results suggest that while cocaine analyte concentrations may be significantly higher in dark hair types, including AA individuals, use of benzoylecgonine /cocaine ratios and extensive decontamination wash criteria greatly reduce positive hair testing results in this *in vitro*

surface contamination model. These findings could have a significant impact on whether national agencies use hair testing.

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LIST OF ACRONYMS

AA	dark-haired African American
BC	light-haired Caucasian
CE	cocaethylene
CE-ESI-MS	capillary electrophoresis-electrospray ionization mass spectrometry
CFS	Center for Forensic Sciences
COC	cocaine
DC	dark-haired Caucasian-
GC-MS	gas chromatography-mass spectrometry
HCl	hydrochloride
HILIC-MS/MS	hydrophilic interaction chromatography-tandem mass spectrometry
IRB	Institutional Review Board
LC-MS/MS	liquid chromatographytandem mass spectrometry
MALDI-MS	matrix assisted laser desorption ionisation mass spectrometry
MeOH	methanol decontamination
NCOC	norcocaine
NIJ	National Institute of Justice
NW	no decontamination wash process
pH 6	potassium phosphate buffer
PI	Principal Investigator
PO4	extended phosphate buffer decontamination wash
RTI	RTI International
SAMHSA	Substance Abuse and Mental Health Services Administration
SEM	scanning electron microscopy
SPE	solid phase extraction
UPLC-TOF-MS	ultra-performance liquid chromatography-time-of flight mass spectrometry

EXECUTIVE SUMMARY

Statement of the Problem

The potential for "bias" from an individual's hair color and/or ethnicity has lead to criticisms of hair testing, including questions of its forensic utility. One individual's exposure to the same drug concentration may have a greater chance of drug detection in their hair than another individual, in part, because of the color of their hair and/or their ethnicity.

This possibility of bias may also extend to the incorporation of drug into hair due to environmental contamination, but research is limited. For an individual (e.g., a narcotics officer) exposed to a drug as a result of their job, this could have serious adverse consequences, including occupational health hazards, job security, and accusation of criminal misconduct. Other criminal justice applications, such as evidentiary support of a drug-facilitated crime or maintenance of parole/probation and other drug compliance programs, are equally affected. Thus, a greater understanding of drug binding in hair is critical.

After more than 20 years of hair drug testing, the issue of a "hair color " or "ethnic" bias remains highly controversial in the literature. The purpose of this study by RTI International's (RTI's) Center for Forensic Sciences (CFS) was to determine if a difference exists in drug binding and retention in diverse hair types. The specific goal was to evaluate cocaine (COC) analytes in hair of different color (e.g., light, dark) and ethnic origin (e.g., Caucasian, African American) after the hair had been subjected to surface COC contamination. Secondary research goals were 1) to evaluate the use of microscopy and staining techniques to assist in predicting drug incorporation into hair; and 2) to evaluate the effectiveness of two commonly used laboratory decontamination washes (e.g., brief methanolic wash and extensive aqueous

phosphate buffer wash) to remove contaminant drug from exterior surfaces following an *in vitro* surface contamination model. RTI used hair from multiple individuals (n=12) in each of three color/ethnicity groups (i.e., light-haired Caucasian [BC], dark-haired Caucasian [DC], dark-haired African American [AA]).

Research Purpose

Interpretations of hair drug testing results are complicated by many issues and require continued research. Two ongoing areas of focus for increasing our understanding of drug incorporation into hair and subsequent hair drug testing are the unequivocal ability to differentiate environmental contamination from drug ingestion, and the potential effects of hair color and ethnicity on drug incorporation into hair. Previously, RTI has used their *in vitro* COC surface contamination of hair to evaluate the concentration ratios using different COC sources, drug-user populations, and surface-contaminated specimens. These studies suggest that the current criteria for COC hair testing in many forensic drug-testing laboratories may not effectively discriminate between COC use and environmental COC exposure. Furthermore, preliminary finding of the *in vitro* surface contamination model indicate that darker hair may retain drug more than lighter hair, but the sample size was too small to evaluate statistically.

Results of this study will improve our understanding of the potential role hair color and ethnicity may play in hair testing and will directly affect policy decisions for forensic applications of hair testing. Widespread acceptance of hair testing has been hindered by the possibility that a hair testing bias could lead to different drug test results for individuals with the same exposure/dosing of a drug.

Research Design and Methods

This study was designed to investigate COC analyte concentrations and their ratios in hair that was contaminated through surface contamination with a pharmaceutical-grade solid COC hydrochloride (HCl) powder. The following are seven stages of research grouped and discussed as sections in this report based on experimental design, sample type and preparation, analysis procedures, findings, and conclusions:

- Stage I: Collection and Analysis of Donor Hair Samples
- Stage II: Characterization of Collected Hair by Microscopy
- Stage III: Surface Contamination of Hair
- Stage IV: Decontamination of Hair
- Stage V: Characterization of Decontaminated Hair
- Stage VI: LC-MS/MS Analysis of Hair Samples
- Stage VII: Analysis of Wash Fractions

During this study, almost 2600 samples were prepared and analyzed by microscopy and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to determine the morphological characterization of hair and the presence of COC, respectively. Sample types are listed in **Table ES-1** by the stage of the study and the number of specimens.

Table ES-1. Number and Analysis of Specimens in RTI Study

Stage of Study	Sample Description and Analytical Procedures	Number of Specimens
Stage I	Collection and LC-MS/MS analysis of donor hair for cocaine	▪ AA: 22 ▪ BC: 13 ▪ DC: 14
Stage II	Microscopy of pre-treated hair ▪ SEM ▪ Freeze fracture ▪ Rhodamine B ▪ Methylene blue ▪ Rhodamine B - bright field ▪ Methylene blue - bright field ▪ No stain - bright field	 ▪ 38 ▪ 38 ▪ 76 (2 stain exposure times) ▪ 38 ▪ 76 (2 stain exposure times) ▪ 38 ▪ 38
Stage III	Surface contamination of hair	▪ AA: 13 ▪ BC: 12 ▪ DC: 12
Stage IV	MeOH decontamination of hair Time points 1-11	▪ AA: 139 ▪ BC: 132 ▪ DC: 132
Stage IV	PO4 decontamination of hair Time points 1-11	▪ AA: 139 ▪ BC: 132 ▪ DC: 132
Stage V	Microscopy of decontaminated hair (TP 4) ▪ SEM ▪ Freeze fracture ▪ Rhodamine B ▪ Methylene blue ▪ Rhodamine B - bright field ▪ Methylene blue - bright field ▪ No stain - bright field	 ▪ 37 ▪ 37 ▪ 74 (2 stain exposure times) ▪ 37 ▪ 74 (2 stain exposure times) ▪ 37 ▪ 37
Stage VI	LC-MS/MS analysis of decontaminated hair	▪ 806
Stage VII	LC-MS/MS analysis of decontamination wash fractions (reference lab)	▪ 220
Total		▪ 2593

Findings

Stage I: Collection and Analysis of Donor Hair Samples

RTI collected hair samples of light and dark colors and different ethnicities under an Institutional Review Board (IRB)–approved protocol. Twelve hair samples were required for each group (i.e., BC, DC, and AA) to have enough power statistical significance to determine if

bias existed. RTI sent portions of the donor hair samples to reference laboratory for drug screening by immunoassay and LC-MS/MS confirmation. Hair samples that were positive for COC were excluded from further use in this study. Forty-nine hair specimens were collected, and 12 were excluded from the study (11 COC positives, 1 hair sample with inadequate sample volume). A total of 37 samples were included and used in the *in vitro* surface contamination model of hair with COC powder.

Stage II: Characterization of Collected Hair by Microscopy

Ultra structure morphology of collected hair was examined by several microscopy techniques before performing any surface contamination of the hair. Scanning electron microscopy (SEM) of the hair was used to determine if consistent structural differences were visible at the ultra structure level. Hair strands and knotted hair samples were examined by SEM to observe the surface of the hair under relaxed and strained conditions. Hair samples were freeze fractured to observe the cuticular thickness and number of cuticular scales. Donor hair samples were subjected to several staining methods and viewing techniques of the stained hair samples to examine hair porosity. Hair samples were stained by rhodamine B at two different exposure times and by methylene blue. The hair samples were examined by incidence fluorescence microscopy and also by bright field microscopy. Hair samples that were not stained were also examined by bright field microscopy.

Stage III: Surface Contamination of Hair with COC HCl Powder

Stage III consisted of contaminating the hair with powdered COC. Hair was contaminated by applying powdered COC (~8 mg/10 g of hair) to gloved hands, dampening the gloves with artificial sweat, and then rubbing the hair with the gloved hands to distribute the drug as evenly as possible over the hair. There were three contamination events throughout the

duration of the study. To simulate normal sweating and body oils, the hair was treated once with a synthetic sweat solution containing a small amount of oil, prior to the first hygienic treatment. Hair samples were washed (shampooed) once each weekday throughout the 8-week study period after contamination to simulate normal hygienic treatment of hair.

Stage IV: Decontamination of Hair

Specimens were removed at 11 time points with respect to contamination (i.e., pre-contamination, 1 hour, 6 hours, 27 hours, weekly) during a 56-day period. At each time point, 3 aliquots of approximately 120 mg each were removed and placed in screw-cap vials. Decontamination of time point samples was performed immediately following sample collection. Aliquots were designated as follows: no decontamination wash process (NW), methanol decontamination (MeOH), and an extended phosphate buffer decontamination wash (PO4). The NW aliquots were collected and stored for later potential analysis. The MeOH aliquots were treated by adding methanol to the vial at a volume to adequately cover the hair, capping the vial, and placing it on a shaker for 8 minutes. The methanol was decanted with a pipette and discarded. The same process was repeated a second time, with the methanol from the second wash being transferred to a clean, labeled vial and stored at -20°C for later analysis.

For the PO4 decontamination, isopropanol and phosphate buffer were warmed in a water bath set at 39°C. The hair in the vial was covered with warm isopropanol, capped, and placed on a warmed shaker for 15 minutes. The isopropanol was removed with a pipette and discarded. Next, the hair was covered with warm (39°C) phosphate buffer using the same vial, placed on the shaker for 30 minutes followed by removal and discarding of the phosphate buffer solution. This procedure was repeated for a total of three 30-minute washes, and then two 60-minute washes. The total washing time of the extended phosphate buffer wash procedure was 3 hours and 45

minutes. At the end of the second hour wash, the buffer was transferred to a clean, labeled vial and stored at -20°C until later analysis.

The results of this study were consistent with what we have previously published for contaminating hair with pharmaceutical-grade COC (Stout et al., 2006). Surface contamination by COC resulted in significant quantities of COC on the hair and remaining on the hair over the course of 8 weeks. As was previously observed, there was a significant decline in the COC content over the course of the study. This study was also consistent with previous findings because the benzoylecgonine/cocaine (BE/COC) ratio increased significantly over the course of the study period.

Stage V: Characterization of Decontaminated Hair

At time points 4, 6, 8, and 10, approximately 20 mg was removed from each sample after decontamination for microscopy analysis. The samples from time point 4 were examined by SEM and the previously described staining procedures. These images were compared to the microscopy images taken of the hair samples before contamination to observe the effect of the decontamination processes on the hair structure.

Stage VI: Analysis of Hair by LC-MS/MS

Decontaminated hair samples were analyzed by LC-MS/MS. Samples were extracted in batches of one time point (both decontamination methods), along with calibrators and control samples. Briefly, samples were prepared by adding an extraction solution of 0.1 M potassium phosphate (pH 2.7) to each tube containing 10 mg of hair. The tubes were capped and vortexed, centrifuged for 3 minutes, and sonicated in a water bath at 65–70 °C for 3 hours. Samples were cooled to ambient temperature before the buffer was decanted and 0.1M potassium phosphate

buffer (pH 6) was added. The tubes were again vortexed and centrifuged before being subjected to solid phase extraction (SPE).

A LC-MS/MS method previously used in our laboratory for the analysis of benzoylecgonine (BE) in urine was optimized for the detection of COC, BE, cocaethylene (CE), and norcocaine (NCOC). The LC-MS/MS equipment consisted of an Agilent 1200 series LC coupled to an Agilent 6410 triple-quadrupole mass spectrometer, with an electrospray source operating in positive mode (Santa Clara, CA).

RTI compared COC analyte concentrations and ratios in contaminated hair to the Division of Workplace Programs of the Substance Abuse and Mental Health Services Administration (SAMHSA)–proposed Mandatory Guidelines criteria for federal workplace drug-testing programs and six other decision points of positive calls on confirmatory analytical results. The additional decision points were selected based on a review of the data and previously proposed criteria (Schaffer et al., 2007). Criteria 1 through 3 are the original criteria proposed in the Mandatory Guidelines. Criteria 4 and 5 evaluated the norcocaine/cocaine (NCOC/COC) ratios of 0.05 and 0.01, respectively. Criteria 6 through 9 evaluated the CE/COC ratios greater than or equal to each of the following: 0.05, 0.02, 0.01, and 0.002. It is important to note that RTI applied these criteria to hair specimens because a reference laboratory would apply them under the proposed Mandatory Guidelines. In other words, the reference laboratory would have had to analyze the specimens and apply the cutoffs directly to these results. These criteria are summarized in **Table ES-2**.

Table ES-2. Cocaine Analyte Concentrations and Ratios Criteria for the Determination of Confirmation Results as Positive or Negative

Criteria 1 (BE criteria)	COC ≥500 pg/mg and BE ≥50 pg/mg and BE/COC ≥0.05
Criteria 2 (CE criteria)	COC ≥500 pg/mg and CE ≥50
Criteria 3 (NCOC criteria)	COC ≥500 pg/mg and NCOC ≥50
Criteria 4	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.05
Criteria 5	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.01
Criteria 6	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.05
Criteria 7	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.02
Criteria 8	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.01
Criteria 9	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.002

Stage VII: Analysis of Wash Fractions

As Schaffer and colleagues (2007) have noted in several publications (Cairns et al., 2004a, b), they have applied various ratios of compounds and used various mathematical calculations using the amounts of a drug found in the last wash solution to assist in assessment of a hair test results. As noted by Kidwell and Smith (2007), this wash criteria has evolved over the years. In addition to the decision criteria applied, the wash criteria as described by Cairns and colleagues (2004b) was also applied to the hair COC results. RTI retained the last wash solutions of all hair specimens and sent a representative portion of the washes to a reference laboratory for analysis.

A decision calculation of multiplying 5 times the COC concentration of the last wash solution in the extended phosphate buffer decontamination and subtracting this from the hair COC concentration was performed. If this calculated result still exceeded the decision criteria, it was a "positive" call. Decontamination washes were collected and analyzed by a reference laboratory at time points 1, 3, and 11 to evaluate the addition of a laboratory wash criterion.

Conclusions

Environmental exposure to drugs continues to be a concern for hair drug testing studies, child protection services, probation and parole, and the criminal justice system. It is essential to the validity of hair drug testing that a drug user can be easily identified in comparison to an individual who has not ingested the drug, but may have been unknowingly or knowingly (e.g., narcotics officer) exposed to a drug in their environment. It is also important to determine: 1) if an individual with a given type of hair color or ethnicity type has a greater susceptibility to a positive hair drug test subsequent to environmental exposure; 2) if hygienic treatments affect the positive hair drug test results; 3) if susceptibility of hair to drug environmental exposures can be qualitatively determined through hair characterization studies, such as morphological analysis using microscopy techniques; and 4) if laboratory decontamination procedures can minimize the effects of environmental exposure enough to properly identify a drug user from an environmental contaminated individual.

The results of this study are consistent with RTI's previously published results for contamination of hair with pharmaceutical-grade COC (Stout et al., 2006b). In the prior study, all three COC sources resulted in significant quantities of COC being present on the hair and remaining there over the course of 10 weeks. The COC analytes were resistant to removal by hygienic treatment or by laboratory decontamination; however, there was a significant decline in the content of COC over the course of the study. Similarly, BE/COC ratios increased significantly over the course of the study period. While the previous study did not investigate the effect of hair color and type with an adequate statistical power, this study used the same *in vitro* surface contamination model to evaluate the effects of hair color and type on COC concentration in hair and laboratory decontamination washes. In addition, the previous study did not evaluate

additional assessment criteria; mainly, the effectiveness of decontamination wash procedures or the use of mathematical wash criterion.

Our current results suggest that the hair characterization studies such as morphological analysis using microscopy techniques could not qualitatively establish a relationship between the susceptibility of hair to drug environmental exposures of drug and morphological features. SEM analysis, bright field light microscopy, and incidence fluorescence microscopy with methylene blue and rhodamine B staining showed that inter-individual variation was large for all hair types and ethnic groups. There were no clear morphological features by any microscopy method used that demonstrated an obvious relationship to ethnicity or to hair color.

In this study, methanolic decontamination was not very effective at removing COC analytes from the surface of the hair following this *in vitro* contamination model. In contrast, the extended phosphate decontamination resulted in significantly lower concentrations of all targets. Within each decontamination method, after methanolic decontamination, AA hair had more COC compared to BC hair ($p=0.015$) and DC hair ($p=0.046$), but DC and BC hair were not significantly different. AA hair had significantly greater CE content than only BC hair ($p=0.0054$), and NCOC was also greater in AA hair than BC hair ($p=0.009$). After phosphate decontamination, AA hair had greater concentrations of COC than BC hair ($p=0.015$), but not DC hair. For CE, again, AA hair had greater concentrations than only BC hair ($p=0.019$) AA individuals had significantly lower BE/COC ratios than BC ($p=0.0007$) and DC ($p=0.015$) after methanolic decontamination and also after phosphate decontamination, though the significance of the difference between AA and DC hair was marginal ($p=0.05$). Overall, the extended phosphate decontamination resulted in far fewer positive hair results at early time points, but it did not entirely eliminate positive results. This *in vitro* contamination model suggests no

relationship of positivity and ethnicity exists in situation of environmental COC surface contamination and exposure.

For the nine decision criteria investigated in this study, it is important to note that RTI applied these criteria to hair specimens because a reference laboratory would apply Criteria 1–3 under the proposed federal Mandatory Guidelines and additional cocaethylene /cocaine (CE/COC) and NCOC/COC ratios were also under investigation as part of this study. In other words, the reference laboratory would have had to analyze the specimens and applied the cutoffs directly to these results to determine if a hair sample was positive for COC. Furthermore, the proposed Mandatory Guidelines (SAMHSA, 2004) do not have a provision for the use of such criteria; but our results indicate that use of such a wash criterion in hair drug testing may be a needed layer of separating a drug-user from a non-user. The application of a decontamination wash and wash criterion may also assist with the elimination of a “color bias” or “ethic bias” because this simple and conservative “clinical or assessment adjustments” may be enough to modify "the threshold values and negate the hair color contribution, as proposed by Mieczkowski and Kruger (2007). Since the outcome of possible multiple biases is not known, further research is needed to determine whether the variables can be adequately controlled and resolved through analytical techniques employed for hair testing for drugs of abuse.

The work performed under this study may directly affect the use of hair testing in a variety of investigatory applications and criminal justice systems. Many of its implications for policy and practice complement prior hair drug testing research at RTI.

The results of this study will influence how hair-testing results are interpreted and could significantly impact whether national agencies continue to use hair testing in their drug-free

workplace programs and criminal justice applications. These results may directly affect policy implementation for drug-facilitated crimes (e.g., drug-facilitated sexual assault, homicide by drug poisoning, child abuse by drug poisoning), and parole and rehabilitation compliance. By contributing evidence through *in vitro* environmental COC contamination studies supporting the use of more stringent laboratory decontamination methods and wash criterion calculation can minimize and even eliminate any potential "color" or "ethnic bias" in hair drug testing.

The proposed Mandatory Guidelines for hair testing may need to be amended by adding an additional specimen preparatory step for COC analysis that would include a requirement for a laboratory to decontaminate hair specimens prior to analysis. Furthermore, potential criteria to evaluate the decontamination solutions in relation to the hair concentrations may be necessary unless the presence of other unique COC metabolites in hair can be reliably established. These laboratory adjustments are very much like administrative concentration cutoffs used in clinical and drug testing laboratories routinely. Until such a time that these multiple biases of an individual's hair are more fully understood, these analytical steps may protect individuals from the issues of environmental contamination and intrinsically greater drug deposition into darker hair.

1. INTRODUCTION

1.1 Background

For more than 30 years, hair has been used as a biological matrix to detect controlled substances, such as cocaine (COC), and to indicate drug use. While matrices such as blood, oral fluid, and urine document an individual's drug exposure, ranging from minutes to days, hair may extend the detection period from months to years, depending on the hair length and anatomical location sampled and the collection process. Conventional matrices may have certain limitation as they be both physically and socially invasive to collect, and also require preservation, including chemicals, refrigeration, and/or freezing. Urine may be susceptible to adulteration or substitution. In contrast, hair is easy to collect, relatively stable, and difficult to adulterate or substitute (Ropero-Miller et al., 2009).

Although hair testing has many forensic applications, such as death and drug-facilitated crime investigations, workplace drug testing, and violation of probation or parole, scientists have not been able to eliminate many issues that limit its widespread use. These issues include the absence of standardized techniques between laboratories and laboratory certification programs; consistent proficiency-testing materials; consistent results between laboratories; easily identifiable drug analytes that discriminate between environmental contamination and drug use; and the presence of a potential bias of drug incorporation of drug into hair (i.e., color or ethnic differences) (Ropero-Miller, 2007; Ventura et al., 2007). The issues of environmental contamination and potential drug incorporation biases of hair have been the primary hindrances for hair drug testing because both may affect result interpretation and the forensic validity of its use in court proceedings.

Hair growth patterns and biological and environmental factors influencing its growth will affect drug incorporation into hair. Factors influencing intra-individual growth rate of hair include age, gender, ethnicity, heredity, climate, health, injury, and physical stress, as well as the anatomical site of hair growth (Myers and Hamilton, 1951; Hold, 1996; Robbins, 2002; Kronstrand and Scott, 2007).

1.2 Statement of the Problem

The potential for bias from an individual's hair color and/or ethnicity has led to criticisms of hair testing, including questions about its forensic utility. One individual's exposure to the same drug concentration may have a greater chance of drug detection in their hair than another individual, in part because of the color of the individual's hair and/or his/her ethnicity.

This bias may also extend to the incorporation of drug into hair due to environmental contamination, but research is limited. For an individual (e.g., a narcotics officer) exposed to a drug as a result of his or her job, this could have serious adverse consequences. Because of the potentially significant ramifications for the criminal justice system and for individuals, including those occupationally exposed, a greater understanding of drug binding in hair is critical.

To date, the issue of an ethnic or hair color bias in hair testing remains highly controversial in the literature. The purpose of this study by RTI International's (RTI's) Center for Forensic Sciences (CFS) was to determine if a difference exists in drug binding and retention in diverse hair types. The specific goal was to evaluate COC analytes in hair of different color (e.g., light, dark) and ethnic origin (e.g., Caucasian, African American) after the hair had been subjected to surface COC contamination. Secondary research goals were 1) to evaluate the use of microscopy and staining techniques to assist in predicting drug incorporation into hair; and 2) to

evaluate the effectiveness of two commonly used laboratory decontamination washes (e.g., brief methanolic wash and extensive aqueous phosphate buffer wash) to remove contaminant drug from exterior surfaces following an *in vitro* surface contamination model. RTI used hair from multiple individuals (n=12) in each of three color/ethnicity groups (i.e., light-haired Caucasians [LC], dark-haired Caucasians [DC], and dark-haired African Americans [AA]).

1.3 Literature Review

1.3.1 Utility of Hair Testing for Detection of Drugs of Abuse

Forensic laboratories analyze hair for controlled substances as a complementary and alternative test matrix to blood and urine. Researchers have studied the disposition of many controlled substances into hair, and hair test results have been used as evidence in civil, criminal, and military courts of law (Huestis, 1996; Stout, 2007b). Controlled substances found in hair include, but are not limited to, amphetamines and methylenedioxy analogs, COC, ethyl alcohol (biomarkers), opiates and synthetic opioids, cannabinoids, barbiturates, phencyclidine, benzodiazepines, and non-benzodiazepine hypnotics (e.g., zolpidem) (Ropero-Miller et al., 2000; Ropero-Miller et al., 2005; Ropero-Miller et al., 2009). Many laboratories use either liquid chromatography-tandem mass spectrometry (LC-MS/MS) or gas chromatography-mass spectrometry (GC-MS) as methods of testing (Ropero-Miller et al., 2009). Recently, Gottardo and colleagues (2007) have used capillary electrophoresis-electrospray ionization mass spectrometry (CE-ESI-MS): Vogliardi and colleagues (2010) have used matrix-assisted laser desorption ionisation mass spectrometry (MALDI-MS): Quintela and colleagues (2010) have used hydrophilic interaction chromatography-tandem mass spectrometry (HILIC-MS/MS); and Nielsen and colleagues (2010) have used ultra-performance liquid chromatography-time-of flight mass spectrometry (UPLC-TOF-MS). Hair testing for controlled substances has been

complicated by many issues, including potential color bias, external contamination, and high individual variability with respect to such factors as age, gender, hygiene, drug biotransformation and excretion, and hair growth rate (Cone, 2001; Ruth and Stout, 2004; Balíková, 2005; Stout, 2007b; Ropero-Miller et al., 2009).

Drug can be deposited on surfaces during ingestion or preparation and subsequently transferred to the hair. Some individuals who do not use drugs may be in "at risk" locations where they encounter drugs in their environment. If a hair color or ethnic bias exists with hair testing for drugs, the same drug exposure may result in a positive drug test for one individual and a negative drug test for another. For example, some research has suggested that an African American narcotics officer working to recover illicit bulk drug materials would be more likely to test positive for the drug than a Caucasian law enforcement officer exposed to the same environment (Hoffman, 1999). In contrast, a recent large-scale epidemiological study by Mieczkowski investigated if there is a departure in rates of positive and negative outcomes when comparing the results of hair analysis to urinalysis (deemed "unbiased test") for COC by racial groups. His findings show that the ratios of each racial group are effectively the same for hair and urine assays; that is, the relative risk and risk estimates for positive and negative outcomes are similar for both racial groups (Mieczkowski, 2010).

Although hair testing has the potential to offer a longer window of detection of an individual's drug use compared to blood and urine, the potential for false-positive results or positive results due to environmental contamination rather than drug use could significantly limit the utility of this matrix. This matrix could lose much of its advantage if it is found to be indefensible in court because of environmental contamination or hair color or ethnicity bias.

1.3.2 Factors That May Contribute to Hair Color/Ethnicity Biases

Ethnicity and Race in the United States: Race and ethnicity, while distinctly defined, historically have been used interchangeably while the concepts continue to evolve even to this day. Race defines one's biologic or genetic heritage to include physical traits such as skin color and hair color, while ethnicity describes one's cultural background or allegiance. The U.S. Census is a good representation of this phenomenon. As Margaret Winker wrote in a 2004 JAMA editorial entitled, "Measuring Race and Ethnicity: Why and How?":

> *Since the 1960 census, individuals have been able to self-designate race; an option for Hispanic identity was added in 1970, and beginning in 2000, respondents could self-designate more than one race category. Self-designated race is particularly important when race is measured to act as a proxy for a social or cultural milieu.*

The 2010 U.S. Census combined the "race" and "ethnicity" categories into one question to appear as "race/ethnicity" for a more meaningful description for the American people (U.S. Census Bureau, 2010, http://www.census.gov/compendia/statab/cats/population/estimates_and_projections_by_age_sex_raceethnicity.html). The 2010 U.S. Census listed 15 race/ethnicity categories, including: 1) White; 2) Black, African American or Negro; 3) American Indian or Alaskan Native; 4) Asian Indian; 5) Chinese; 6) Filipino; 7) Japanese; 8) Korean; 9) Vietnamese; 10) Other Asian; 11) Native Hawaiian; 12) Guamanian or Chamorro; 13) Samoan; 14) Other Pacific Islander; and 15) Some Other Race. A separate question asks for Hispanic Identity. About 6.8 million (2.4%) of Americans identified with more than one race in the 2000 Census; the percentage was higher for children and young adults and will likely increase in 2010 (Jones and Smith, 2001).

Many studies have indicated that incorporation of drug into hair is proportional to the darkness of the hair. Individuals with darker hair have a higher concentration of melanin pigment, and a melanin affinity for basic drugs in hair is directly proportional to drug incorporation (Balikova, 2005). The toxicology, criminal justice, and legal communities are concerned that this potential color bias may have a disparate impact on particular ethnic groups such as African American, Asian, and Hispanic; all have darker hair and higher melanin content (Caplan and Huestis, 2007).

Several physiological and environmental factors may contribute to a hair color and/or ethnicity bias. These potential contribution factors may be intrinsic or self imposed and may vary widely among individuals, including individuals with the same hair color or in the same ethnic group, and even in the same individual because of variation in hair strands. Researchers have proposed many of these variables as sources of hair color/ethnicity biases; however, a clearer understanding of the contributions of each is needed to assess implications for forensic drug testing. For example, one factor may affect an individual or a particular ethnic group by causing more drug to bind in the hair, while different factors may have a similar effect on another individual or group. The resulting biases may not have an overall effect on the hair test results because they may negate one another; therefore, legal implications of each of these factors would be minimal. If there is a small effect, a simple clinical or assessment adjustment, such as modifying the threshold values, could negate the hair color contribution (Mieczkowski and Kruger, 2007). Since the outcome of possible multiple biases is not known, further research is needed to determine whether the variables can be adequately controlled and resolved through analytical techniques employed for hair testing for drugs of abuse. A review of the scientific literature reveals three factors most often proposed for affecting drug binding in hair: structural

components, pigmentation, and chemical treatments. While other factors may contribute to hair color/ethnicity biases, this study focuses on these three, as discussed below.

Structural Components: Hair is an extension of the epidermal layer of skin that begins at the hair follicle. Its chemical composition includes 65–95% proteins, 1–9% lipids, 0.1–5% melanin pigments, and trace amounts of polysaccharides, water, and essential elements (Kronstrand et al., 1999). Human hair is formed from at least two hair cell types and, potentially, a third.

Overlapping hair cell scales form the outermost layer, the cuticle. Next, the cortex is composed of spindle-like cortical cells. In the core of the cortex, condensed cells, either continuous or interspersed with air pockets, form the medulla, which may or may not be present. Morphological parameters of hair, including color, shape, presence of medullar region, degree of curliness, and thickness, differ among individuals. Caucasians have the greatest variability (Potsch, 1996). Ethnicity influences the thickness of hair fibers. For example, the number of layers of each component affects the thickness of a hair fiber. While Hold reported that the number of cuticular layers could be as high as 8 for African American and 10 for Caucasian hair samples, subjects in this study had a slightly lower hair cuticle layer count at 5 for both African American and Caucasian (Hold, 1996). Fine hair commonly associated with Caucasians can also lack a medullar region altogether. Hair fibers of African American and Asian origin may be heavily medullated, which also contributes to the thickness of the hair fiber (Potsch, 1996). The thickness of hair fibers is strongly influenced by race, with thickness in Caucasians ranging from 47–63 μm, Ethiopian hair 51–90 μm, and Mongolian (Navajo and Mayan) hair fiber thickness ranges from 61–79 μm (Robbins, 2002).

Melanin and Other Matrix Proteins: In humans, hair, skin, and the iris of the eye contain melanin, pigments typically produced by specialized epidermal cells called melanocytes. Melanocytes, which have melanosome organelles that synthesize melanin, are located in the dermal papilla area of the hair bulb and are transferred to migrating keratinocytes that move into the hair fiber during the hair growth. Hair grows on average 0.3–0.4 mm/day, or 1 cm/month, in a cyclic pattern of anagen phase of active growth, followed by regression in the catagen phase and the resting, or telogen phase (Kronstrand et al., 1999). The size of the melanin granules in black hair is up to two times larger than that in brown hair and many times greater than that in lighter hair. Melanin content is highest in Asian populations, followed by African Americans, then Caucasians. The shape of melanin granules varies from elongated in dark hair to spherical in red hair (Potsch, 1996). Melanin pigments are associated with the cortex and medullar regions of the hair more often than the cuticle.

Research has demonstrated that drugs preferentially associate with melanin in hair. **Table 1-1** shows some of the research investigating the mechanism of drug binding to melanin. Similarly, Debing and colleagues (1988), after assessing 15 drugs for their binding capabilities to calf-eye melanin, discussed their study's implications on drug toxicity and pharmacokinetics in light of the tendency for some drugs to bind more avidly to melanin than others. All of these studies demonstrate higher drug concentrations as melanin content—specifically eumelanin content—increases. Eumelanin is responsible for brown and black pigmentation. Yellow and red pigmentations are derived from pheomelanins. Although it is evident that many drugs preferentially bind to melanin, and that darker hair may as a result contain higher concentrations of such drugs, it is unclear how this correlation influences the interpretation of results for a population and whether or not this correlation results in a "racial" or ethnic bias based on color

and hair type. Some researchers have suggested that hair drug testing manifests a bias toward individuals with darker hair because these individuals have more melanin in their hair and, consequently, more drug (Joseph et al., 1996; Rothe, 1997; Wilkins et al., 1998; Rollins et al., 2003). These suggestions imply that ethnic groups with predominantly dark hair, such as Asians and African Americans, are subject to a high bias in hair test results.

Table 1-1. Literature Summary of Publications Examining Drug Binding to Melanin

Author	Date	Study Design	Binding Component	Drugs Investigated	Decontamination	Correlation Drug/Binding Component Content	Correlation Drug/ Hair Color
Borges et al.	2003	Five synthetic melanin subtypes with varying amounts of monomer units (eumelanin or pheomelanin type)	Melanin	Cocaine, benzoylecgonine (BE), amphetamine, N-acetylamphetamine (N-AcAP)	Not reported	Dependent on type of melanin - no drug bound to pure pheomelanin. Also dependent on drug - cocaine and amphetamine showed correlation but BE and N-AcAP did not	N/A (no hair tested)
Joseph et al.	1996	Human	Melanin	Cocaine	Not reported	Direct correlation	Direct correlation
Joseph et al.	1997	Human n = 156	Melanin (hair)	Cocaine	3x 10 ml tap water	Direct correlation	Direct correlation
Kronsdtrand et al.	1999	Human n=9	Melanin	Codeine	Isopropanol:water (2:1)	Direct correlation	Eumelanin has higher bidning capacity than pheomelanins
Kronsdtrand et al.	2003	Human n=16, 9(pigmented and nonpigmented grey haired)	Melanin	Desmethylselegiline, methamphetamine, amphetamine	Not reported	Direct correlation	Direct correlation
Lindquist and Ullberg	1972	Mice (pigmented and albino), monkeys	Melanin (eyes, skin, hair, ear, other organs)	Chloroquine and chlorpromazine	Not reported	Direct correlation	Tested pigmented and albino - don't know if there was a range of color of pigmentation
Lyden et al.	1982	Mice (pigmented and albino)	Melanin (eyes, liver, kidney)	Haloperidol	Not reported	Direct correlation	Tested pigmented and albino - don't know if there was a range of color of pigmentation
Mieczkowski and Kruger	2007	Human hair - black and brown (n = 8687)	Melanin	Cocaine, benzoylecgonine (BE)	Isopropanol, followed by 5 phosphate buffer washes	Mixed effect	Significant difference for BE, but not for cocaine. Effect is very small and can be accounted for by modifying threshold
Potsch et al.	1997	Tortoise-shell guinea pigs n=7	Melanin (hair)	Codeine	3x 5 min in MeOH	Direct correlation	Eumelanins play more important role in drug binding than pheomelanins
Salazar et al.	1978	Human (brain tissue)	Melanin	Chlorpromazine, imipramine, haloperidol	Not reported	Direct correlation	N/A (no hair tested)
Slawson et al.	1998	Mice n=35	Melanin	Phencyclidine	Not reported	Direct correlation	Closely related to the concentration of eumelanin rather than pheomelanin

Other authors have sought to provide evidence that refutes the potential of an ethnic or color bias in hair testing (Kelly et al., 2000). These studies were of various designs, but all were based on statistical or other mathematical *post hoc* analysis of data sets, rather than on controlled studies. Some studies (Mieczkowski et al., 2007; Mieczkowski, 2010) examined several large population samples drawn from a variety of populations, including potential or current police officers and truck drivers. The application of rigorous statistical models to these populations indicates that no ethnic or color bias exists. It may be that the well-demonstrated association of drugs with melanin in more controlled studies of smaller populations is masked by other demographic variability found in the larger populations investigated by mathematical *post hoc* analysis (Schaffer at al., 2005; Hill et al., 2008). Moreover, these statistical models may be confounded by the lack of controlled dosages and by the self-reported ethnic designation in the population studies. Thus, as a result of the high intrinsic variability in hair testing and the relatively small population samples used in the statistical analyses (for the large variation evident in most populations), the issue of incorrectly categorized subjects may skew the results.

Drugs are also associated with the structural proteins of the hair matrix. Studies indicate that drug incorporation still occurs even if the hair has little or no melanin. For example, white hair or albino hair still has the ability to retain drug (Wilkins et al., 1998; Kronstrand et al., 1999). On average, graying, or age-related human hair color loss, begins after 35 years of age, when the pigmentation contributed by the hair bulb melanocytes is lost (Van Neste and Tobin, 2004). At first, graying is the result of a mixture of pigmented and non-pigmented hair; eventually, total loss of pigmentation occurs. Support for drug binding to other structural proteins besides melanin was reported by Stout and Ruth (1998) when they determined that fluorescence due to rhodamine dye sequestering in the cortex region of mouse hair appeared to

be related to other structural proteins. Their study, however, was equivocal for melanin binding, as they could not rule out melanin quenching the rhodamine and fluorescein fluorescence. Similarly, Appelgren and colleagues (1997) employed Scatchard analysis in their *in vitro* studies investigating clenbuterol binding to melanin and keratin. They concluded that clenbuterol distinctly binds to keratin and must play a role in the mechanism of binding that is separate from melanin binding.

Chemical Treatment: Chemical treatments of hair constitute a multibillion-dollar business in America (First Research, Inc., 2007). Hygienic and cosmetic treatments that affect hair fibers include shampoos, conditioners, relaxers (straighteners), hair dyes, chemicals used for permanent waves, styling products such as oil moisturizers and pomades, and styling techniques such as blow-drying and the use of curling or straightening irons. These products can affect the overall health conditions of hair (e.g., brittleness, retention/loss of cuticle, and structural components, including porosity, defined/amorphous, breakage), as well as hair's water content and strength. Hygienic treatment of hair varies widely among individuals; some may wash and condition their hair daily, while others may do so much less frequently. The frequency of these treatments may contribute to the effect of chemical treatments on hair structure. For example, permanent waves commonly used to curl the hair of Caucasians, and straightening products used for African American hair, both use alkaline agents such as sodium/lithium hydroxide or guanidine hydroxide. These harsher chemical treatments are used, on average, more often by African Americans (every 4–8 weeks) than by Caucasians (every 3–6 months) (Bernard et al., 2002; Miller, 2002; de Sa Dias et al., 2007). An important point to remember when considering the impact of hygienic and chemical treatments on drug testing of hair is that hair treatments affect drug binding and retention in both *in vivo* and *in vitro* situations. Polla and colleagues (2009)

reported a case involving a male who repeatedly produced positive hair tests over a period of 15 months and only produced a negative hair test after bleaching his hair.

1.3.3 Results of Previous RTI Investigations Supporting This Study

RTI was previously awarded a National Institute of Justice (NIJ) grant (2006-DN-BX-K019) to investigate COC analyte concentrations in hair after surface contamination, normal hygienic treatment, and multiple laboratory decontamination procedures. In an earlier study, we investigated surface contamination from multiple sources of COC, using one light and one dark hair sample. However, the study was not designed with the statistical power necessary to investigate the effects of hair color or ethnicity. Study results suggest that dark hair incorporates COC at higher concentrations than light hair, and that both hair types retain COC for more than 1 month following surface contamination and laboratory decontamination with an extended aqueous buffer decontamination wash (Ropero-Miller and Stout, 2009; Ropero-Miller, 2011). In addition, RTI previously performed and published results of a similar surface-contamination study (Stout et al., 2006) that was not designed to assess a contamination model for use in the production of performance testing samples. To characterize the effects of decontamination on hair as a preliminary investigation, RTI used scanning electron microscopy (SEM) to evaluate the surface of the hair after decontamination wash procedures were performed. These SEM micrographs indicated that the cuticle of hair subjected to the extended aqueous buffer wash had a dramatic loss of cuticle scales from some hair samples, in comparison to brief and less-harsh methanolic decontamination procedures or the untreated hair itself.

These foundational studies have provided RTI with valuable experience in hair sample preparation and analysis, as well as information about within-individual variation and potential differences in hair types. We used this knowledge and experience in performing this study. This

study assessed different hair types while standardizing variables with respect to contamination, hygienic treatment, decontamination, COC source, and hair testing analyses, and we evaluated the potential impact of ethnicity on external contamination. Our study design used controlled contamination and decontamination protocols, varying only hair color and/or ethnicity, and included a sufficient number of subjects to determine statistically significant differences in drug concentrations between light hair and dark hair or between Caucasian hair and African American hair. Furthermore, two different decontamination protocols were performed on these hair samples to determine if different decontamination protocols affect the magnitude of a potential hair color or ethnic bias.

1.3.4 Contamination Studies in the Literature

Several researchers have investigated procedures for removing COC from hair after external application. Specifically, Romano and colleagues (2001) contaminated the hair of four individuals with powdered COC and found that COC could not be removed from the hair by decontamination procedures. Following a single contamination event, hair samples from these individuals were collected and tested weekly. The subjects were allowed to wash their hair using their normal procedures throughout the study period. After 10 weeks, measurable concentrations of COC were still present in the hair of all test subjects. A review of the literature indicates that aggressive washing techniques can remove COC from hair when performed 1 hour after the drug has been applied to the hair, either as a solution or as a powder (Romano et al., 2001; Schaffer et al., 2002; Stout et al., 2006). However, studies also have shown that wash procedures performed more than 1 hour after drug application do not remove all of the COC from hair (Romano et al., 2001). Cairns and colleagues (2004b) used a solution of COC in chloroform to contaminate study hair; arguing that this more closely approximated superficial (surface) contamination of

hair than COC in aqueous solution because drug in an aqueous solution penetrates the hair shaft. Cairns and colleagues (2004b) stressed differences in the wash procedures used by their group and those used in the study by Romano and colleagues (2001) to explain the differing study results. These investigators reported that, following several shampoo wash-dry cycles, external contamination could be differentiated by an extensive wash and application of a mathematical factor (Schaffer et al., 2002; Cairns et al., 2004a; Hill et al., 2008). Tsanaclis and Wicks (2008) also attempted to use mathematical calculations to distinguish between external contamination and drug use. Hill and colleagues (2008) used methylene blue staining to examine porosity of the hair and found that higher porosity, which causes a high amount of staining, correlates with a high amount of drug uptake. They found that while more porous hair took up more COC, the drug was also more easily removed by the aqueous washes.

In October 2006, RTI published results of a study using powdered COC contamination of hair. The study indicated that (1) external COC contamination may be difficult to remove by decontamination, and (2) COC contamination may be difficult to discriminate from consumption by the presence of certain metabolites. Our work has corresponded closely with that of Romano and colleagues (2001). Significant concentrations of COC were observed in hair samples over the entire study period from a single contamination event. RTI presented the ratio of benzoylecgonine to cocaine (BE/COC) over the study period from hair decontaminated with an extensive buffer wash performed by RTI. The BE/COC ratio significantly increased over the study period ($p<0.0001$) and increased above the 0.05 value proposed for hair testing in federal workplace drug testing programs (SAMHSA, 2004). There was no apparent relationship between the rate of drug loss and extent of BE/COC ratio increase and hair color.

That study showed that contamination of the surface of hair may result in the incorporation of analytes into the hair without the addition of liquid. We observed that samples decontaminated with an aggressive phosphate buffer procedure 1 hour after contamination, and prior to contact with any moisture, were completely decontaminated (no detectable COC or metabolites). Samples taken at the same time, 1 hour after contamination and prior to contact with any moisture, were packaged for shipment to testing laboratories for decontamination and were found to contain significantly more COC analytes, after the COC had been allowed to sit on the surface of the hair for an extended time before decontamination. Comparisons of recent literature discussing hair testing for COC show wide variations in decontamination methods being used, ranging from no decontamination (Garcia-Bournissen et al., 2009; Nielsen et al., 2010) to multiple steps, using either organic washes (Hoelzle, 2008; Bucelli et al., 2009) or a mixture of aqueous and organic washes (Cordero and Paterson, 2007; Schaffer et al., 2007; Barroso et al., 2008; Lopez et al., 2010; Paterson et al., 2010; Quintela et al., 2010).

Many studies have performed hair contamination by different methods, such as, rubbing with drug powder (Stout et al., 2006; Hill et al., 2008), soaking hair in COC solutions of chloroform (Cairns, 2004b), and soaking hair in solutions of drug-containing blood (Paterson et al., 2010). Researchers continue to debate which method is most realistic and efficient.

1.4 Rationale for the Research

Interpretations of hair drug testing results are complicated by many issues requiring continued research. Two continued areas of focus for increasing our understanding of drug incorporation into hair and subsequent drug testing are the unequivocal ability to differentiate environmental contamination from drug ingestion and the effects of hair color and ethnicity on drug incorporation into hair. Previously, RTI has used their *in vitro* COC surface contamination

of hair to investigate the concentration ratios using different COC sources, drug-user populations, and surface-contaminated specimens. These studies suggested that the current criteria for COC hair testing in many forensic drug-testing laboratories may not effectively discriminate between COC use and environmental COC exposure. Furthermore, preliminary findings of the *in vitro* surface contamination model indicated that darker hair may retain drug more than lighter hair, but the sample size was too small to evaluate statistically.

The results of this study will improve our understanding of the role hair color and ethnicity play in hair testing and will directly affect policy decisions for forensic applications of hair testing. Widespread acceptance of hair testing has been hindered by the possibility that a hair testing bias could lead to different drug test results for individuals with the same exposure/dosing of a drug.

2. RESEARCH DESIGN AND METHODS

The purpose of the hair contamination was to investigate the ratios and dynamics of the drug detected in hair after known contamination of hair from each ethnicity. Once the hair was contaminated, RTI sought to determine if there were any significant differences between each of the ethnic groups or between the two decontamination protocols used. The following are the seven stages of research based on experimental design, sample type and preparation, analysis procedures, findings, and conclusions:

- Stage I: Collection and Analysis of Donor Hair Samples
- Stage II: Characterization of Collected Hair by Microscopy
- Stage III: Surface Contamination of Hair
- Stage IV: Decontamination of Hair

- Stage V: Characterization of Decontaminated Hair
- Stage VI: LC-MS/MS Analysis of Hair Samples
- Stage VII: Analysis of Wash Fractions.

RTI used a contamination model that was previously published for this experiment with a 20% reduction in the amount of COC surface contamination amount (0.8 mg/g vs. 1.0 mg/g previous COC amount). Since our quantity of COC used in our *in vitro* contamination model has been criticized by Schaffer and colleagues (2007) as being too high and little information is available on the amount of a drug that exists on surfaces where drug use or handling has occurred, we reduced the amount while keeping the amount at a reasonable size for handling and weighing. Some research has addressed the potential surface contamination of currency (Jenkins, 2001), the potential exposure of police based on a self-report (Mieczkowski and Lersch, 2002), and the exposure of personnel handling large quantities of a drug, as determined by personal breathing space measurements and urine assays (Stout et al., 2006). However, these studies do not provide sufficient evidence about a given drug's potential surface exposure amounts.

The National Jewish Medical Center in Denver, CO, conducted several studies to estimate the exposure potential of workers who process and cleanup sites where methamphetamine has been manufactured. Although the main goal of these studies was to estimate the occupational hazards faced by these workers, the investigators collected surface specimens from known methamphetamine cook sites, and they also conducted controlled methamphetamine cooks to verify the numbers that were obtained from illicit sites (Martyny et al., 2007). Martyny and colleagues (2007) reported the following:

> *Surface contamination throughout the buildings used to manufacture methamphetamine was a consistent finding. Even labs that had been shut down several months prior to testing had high contamination levels of methamphetamine present on many surfaces within the building. Samples as high as 16,000 μg/100 cm^2 were found in the actual laboratories, and a median sample amount of 28 μg/100 cm^2 was detected.*

In a similar study, the group also sampled carpeting up to 20 feet outside the cook area sites and found substantial amounts of methamphetamine (up to 12.4 μg/100 cm^2), indicating that methamphetamine could easily be tracked away from the cook site (VanDyke et al., 2009). Most states consider a structure to be contaminated with methamphetamine unless remediated to low amounts less than 0.1 μg – 0.5 μg/100 cm$_2$ (Martyny, 2008b). The group also sampled personal protective equipment on personnel before and after decontamination and found that wet decontamination procedures may move contamination onto the individual's body (VanDyke et al., 2009). Specimens taken after the personnel were decontaminated revealed that detectable levels of methamphetamine (0.02 μg/4x4 sample wipe) were still present on the personal protective equipment and on the subjects themselves. Generally, two portions of the body were wiped, an area to the front of the body that may be influenced by splashes and contact (face, arms, and hands), as well as the back or neck, which is primarily influenced by airborne exposures. These simulated methamphetamine cook studies suggest that even after a clandestine laboratory ceases operation, a number of compounds, including the drug itself, may persist (months to years) after the cook has been completed (Martyny, 2008a). These findings for methamphetamine demonstrate that there is a significant contamination potential in illicit drug use sites. Methods of manufacturing COC and methamphetamine are different, but the extensive

contamination potential with methamphetamine suggests that COC surface contamination may be extensive as well in locations where use or handling of COC has occurred.

It is crucial to the legal acceptance of hair-testing results that it be possible to differentiate environmentally exposed individuals from drug users, given current analytical technologies and results interpretation. Additionally, it is critical to understand if there is a potential for African American hair to be more sensitive to the accumulation or retention of environmental COC than Caucasian hair.

2.1 Experimental Design — Contamination of Hair with Pharmaceutical Cocaine

2.1.1 Subject and Sample Collection Criteria

For the contamination studies, head hair was obtained from human volunteer donors from each of the ethnicity groups. A total of 49 hair samples were collected for this study. RTI collected hair samples from 39 donors directly under an Institutional Review Board (IRB)–approved protocol that includes informed consent of the donors. Subjects were recruited into each group until at least 12 individuals were collected and the hair was determined to be drug free. Exclusions of individuals from this study using the IRB–approved protocol included children under the age of 7 (less ability to understand research study and consent to participation); subjects that did not meet the hair color criteria (requirement of dark brown to black and light blonde); adequate volume of head hair (approximately 10 grams); color treated hair; and known exposure to COC. No personally identifiable information was collected or retained on any donor.

At the time of collection, IRB consent forms that required the donor's name and signature (identifiable information) were completed, but kept separate from donor hair samples. A

collection ID number was assigned to each donor hair (numeric date and alpha sequence order of collection for a given day; January 1, 2010, first hair specimen collected given a collection ID of 010110A). To assist with blinding samples to analysts, a subsequent Accession ID and Contamination ID were also assigned to a subject's hair to assist with documenting processing of the sample through the necessary laboratory procedures. Donors were solicited through word-of-mouth inquiries and were compensated for donations. Prior to hair collection, each donor met with this study's principal investigators (PIs) to discuss the research purpose and design, review the IRB forms, and complete a series of interview questions to determine donor eligibility. Minors (under the age of 18 years) had to have a legal guardian present, and both the subject and the legal guardian participated in the process and signing of the IRB forms. Questions were as follows:

- Would you be interested in participating, and can I ask you a couple of preliminary questions to see if you are eligible to participate?
- Do you have any potential exposure to cocaine, either through usage or in your environment, such as a family member who uses cocaine, or a job such as law enforcement that might put you near cocaine?
- Have you colored or bleached your hair recently?
- What is your age?
- What ethnicity do you associate yourself with (African American, Caucasian, Hispanic, other)?
- What cosmetic treatments have you done to your hair recently, such as permanent waves, or chemical straightening?
- How often do you wash your hair?

- In a majority of cases, do you let your hair air dry or use a dryer?

All donations were collected by the Principal Investigators (PIs) by either cutting pony tails or using a cleaned electric shaver. Examples of each IRB form are included in Appendix A. For the remaining ten donors, hair samples were obtained from RTI's hair inventory, which was established prior to this study, or purchased from a commercial source. Procedures for collection, demographics, and inclusion criteria were less restrictive (i.e., age less than 7 years accepted) and complete for these donor collections.

After evaluating hair subjects for inclusion criteria, a total of 12 subjects from each of the ethnic groups were collected. A total of 12 donor hair samples were excluded from this study due to positive drug screening results (see results section). Subjects were placed in an ethnic group based on self identification and corroborating observation. Hair color was determined initially by visual observation by the PI during subject interview and hair collection and subsequently assigned a hair color based on two independent corroborating observations using a Schwarzkopf Natural Selections color scale as described in **Table 2-1** (Schwarzkopf, 2001).

Table 2-1. Hair Characterization by Schwarzkopf Natural Selections Color Scale

Schwarzkopf Color	
1.0	Black
2.0	Light Black
3.0	Dark Brown
4.0	Medium Brown
5.0	Light Brown
6.0	Dark Blonde
7.0	Medium Ash Blonde
8.0	Light Ash Blonde
9.0	Extra Light Ash Blonde
10.0	Ultra Light Ash Blonde

The date of receipt, Schwarzkopf color, and demographic information were recorded for each donor hair. Most subjects washed their hair on a daily basis, with African Americans washing less frequently (typically on a weekly basis). Also, most subjects let their hair air dry as opposed to hair dryer use, with African Americans blow drying less frequently. The three ethnic groups were defined as blonde Caucasian (BC) group, identified as a Schwarzkopf color of 8–10; dark brown or black Caucasian (DC), identified as a Schwarzkopf color of 1–5; and dark brown or black African American (AA), identified as a Schwarzkopf color of 1–3. Twelve individuals in each group were used to balance the costs of collection and the statistical power in each ethnic group. **Table 2-2** provides demographic information collected and descriptions of the donor hair for each of the individuals used in the study.

Table 2-2. Hair Donor Information – Demographics, Collection Type and Color Characterization

Collection ID	Accession ID	Contamination ID	Gender	Ethnicity	Study Group	Age	Type of Collection	Schwarzkopf Scale
Sample #1	11367-150-1	11367-152-1	Male	AA	AA	32	Shaving	1
Sample #2	11367-150-2	11367-152-2	Male	AA	AA	17	Shaving	1
Sample #3	11367-150-3	11367-152-3	Male	AA	AA	65	Shaving	3
11612-28-1	11367-150-4	11367-152-4	Male	AA	AA	unknown	Shaving	5
072309A	11367-150-7	11367-152-7	Female	AA	AA	40	Cut sheers	3
071409A	11367-150-9	11367-152-9	Male	AA	AA	44	Cut sheers	1
071409F	11367-150-10	11367-152-10	Female	AA	AA	29	Cut sheers	1
072009B	11367-150-13	11367-152-13	Female	Caucasian	BC	19	Cut scissors	9
072209B	11367-150-14	11367-152-14	Male	Caucasian	BC	9	Razor	8
072209A	11367-150-15	11367-152-15	Male	Caucasian	BC	8	Razor	8
072309B	11367-150-16	11367-152-16	Male	Caucasian	BC	20	Razor	10
072709A	11367-150-17	11367-152-17	Male	Caucasian	BC	9	Razor	9
071409K	11367-150-18	11367-152-18	Female	Caucasian	BC	11	Cut sheers	9
071709B	11367-150-19	11367-152-19	Female	Caucasian	BC	34	Cut	9
071309C	11367-150-20	11367-152-20	Female	Caucasian	BC	12	Cut sheers	9
11367-22-1	11367-150-21	11367-152-21	Female	Caucasian	BC	13	Cut	10
11367-22-3	11367-150-22	11367-152-22	Female	Caucasian	BC	5	Cut	10
11367-22-5	11367-150-23	11367-152-23	Female	Caucasian	BC	5	Cut	10
10772-50-1-4	11367-150-24	11367-152-24	Female	Caucasian	BC	13	Cut	9
071409E	11367-150-25	11367-152-25	Female	Caucasian	DC	35	Cut sheers	4
071409C	11367-150-26	11367-152-26	Female	Caucasian	DC	22	Cut sheers	3
071409L	11367-150-27	11367-152-27	Male	Caucasian	DC	11	Razor	4
071409G	11367-150-28	11367-152-28	Female	Caucasian	DC	32	Cut sheers	4
071709A	11367-150-29	11367-152-29	Male	Caucasian	DC	39	Razor	3
071509A	11367-150-30	11367-152-30	Female	Caucasian	DC	11	Cut sheers	3
072109A	11367-150-31	11367-152-31	Male	Caucasian	DC	31	Razor	4
070609A	11367-150-33	11367-152-33	Male	Caucasian	DC	22	Razor	4

(continued)

Table 2-2. Hair Donor Information – Demographics, Collection Type and Color Characterization (continued)

Collection ID	Accession ID	Contamination ID	Gender	Ethnicity	Study Group	Age	Type of Collection	Schwarzkopf Scale
071409B	11367-150-34	11367-152-34	Male	Caucasian	DC	47	Razor	4
11612-28-2	11367-150-36	11367-152-36	Male	Caucasian	DC	Unknown	Shaving	4
072109B	11367-150-37	11367-152-37	Male	AA	AA	43	Razor	1
122309A	11367-150-41	11367-152-41	Male	Caucasian	DC	13	Electric clippers	3
012910B	11367-150-43	11367-152-43	Female	AA	AA	Unknown	Cut	6
012910A	11367-150-45	11367-152-45	Female	AA	AA	Unknown	Cut	5
None assigned	11367-150-46	11367-152-46	Male	Caucasian	DC	42	Razor	4
012610B	11367-150-47	11367-152-47	Male	AA	AA	Unknown	Razor	1
071310B	11367-150-49	11367-152-49	Male	AA	AA	18	Razor	1
071410A	11367-150-50	11367-152-50	Female	AA	AA	34	Cut	1

To remove styling materials or other loose dirt prior to any additional processing, each hair was submerged in a beaker of de-ionized (DI) water and massaged for 15 minutes. The water was drained, and the procedure was repeated two more times. The hair was placed between two pieces of filter paper and allowed to dry. Two aliquots of approximately 50 mg each were removed for characterization by microscopy. Approximately 100 mg was removed from the donor hair samples and sent to a reference laboratory for testing to determine if hair was negative for COC analytes and other common drugs of abuse. Following drug-screening analysis, it was determined that additional donors were required to have the desired number of donor in each ethnic group. Donor hair samples 11367-150-48 through 11367-150-50 were collected at a later date and tested by RTI's newly validated in-house LC-MS/MS method instead of being sent to the reference laboratory.

2.1.2 Morphological Characterization of Hair

RTI categorized the hair samples based or color and ethnicity using the Schwarzkopf scale of hair color (i.e., visual inspection) (Schwarzkopf, 2001) and ultra structure morphology evaluated via SEM (Stout et al., 2006; 2007a). SEM of the hair was used to determine if consistent universal structural differences are visible at the ultrastructure level. Representative portions of hair strands and manually knotted portions of hair strands were mounted onto an adhesive carbon substrate on SEM stubs. Five strands per individual were randomly selected and knotted. Each strand was observed, and three strands per individual were imaged at the knot and at the straight portions and along the shaft. Unusual features, if observed, were also imaged. Samples were coated with a layer of evaporated gold-palladium to enhance conductivity, and placed in a FEI Quanta 200 environmental (Hillsboro, OR) scanning electron microscope for analysis. The samples were examined at an appropriate excitation (e.g., 15 kV) to observe

ultrastructure, morphology, and surface features. Relevant features were micrographed at a variety of magnifications, typically from 500x to 5000x. Knotted hair samples were examined to observe the condition of the hair under stress and strain.

Hair samples were also subjected to freeze fracturing followed by examination by SEM to characterize hair samples by number of cuticular layers and cuticle thickness. Single strands of hair were cut to 1.5 cm, then held at each end with forceps and dipped into liquid nitrogen for 5–10 sec. The hair was snapped in half while submersed in the liquid nitrogen, then mounted with fractured ends pointing toward the center. This process was repeated until six fractured hair samples were mounted for each individual.

Additionally, hair samples were stained with Methylene blue based on the method described by Hill and colleagues (2008) and with rhodamine B using the method described by Stout and colleagues (1998). The methylene blue staining was performed by placing hair cut into 1.5 cm lengths onto a slide and pipetting 50 μL of a 0.5% methylene blue solution onto the hair. The hair samples were allowed to soak in the staining solution for 5 minutes, then removed and rinsed in a beaker of DI water for about 5 seconds, or until no more stain could be seen washing off the hair. Once dry, the hair samples were then placed parallel on a clean slide, about 0.5 cm apart. Permount was pipetted on top of the hair samples, and then they were covered with a coverslip. Samples were stored protected from light overnight to allow the Permount to dry before observation.

For the rhodamine B staining, a 1.0 mg/mL stock rhodamine solution was prepared by dissolving 20 mg of rhodamine in 20 mL of pH 6 phosphate buffer. The stock solution was then diluted 1/10 to make a 0.1 mg/mL working solution. Each hair sample to be stained was cut into

1.5 cm lengths and placed on a slide, then covered with 50 µL of 0.1 mg/mL rhodamine solution. The hair samples were left in the staining solution for 5 minutes, then rinsed in a beaker of DI water until no stain could be seen washing off. Once dry, the hair samples were then placed parallel on a clean slide, about 0.5 cm apart. Permount was pipetted on top of the hair samples and then they were covered with a coverslip. Samples were stored protected from light overnight to allow the Permount to dry before observation. The rhodamine staining procedure was performed twice for each hair—first with a staining time of 5 minutes, then with a staining time of 1 hour.

Hair samples were stained with rhodamine B and methylene blue before being examined by bright field microscopy using a BX51 Olympus Microscope (Center Valley, PA). Incidence fluorescence microscopy was performed using an Olympus IX71 scope (Center Valley, PA) equipped with CY3 and CY5 filter sets. RTI compared the hair samples before and after the hygienic and laboratory decontamination procedures to evaluate whether hair samples of different ethnic origin respond differently to these treatments.

2.1.3 Reagents and Analytes for Contamination and Decontamination

All solvents used were analytical-grade solvents and reagents. Methanol, methylene chloride, and ammonium hydroxide were purchased from EMD (Gibbstown, NJ). Isopropanol was purchased from Burdick and Jackson (Muskegon, MI). Potassium phosphate was purchased from BDH (VWR West Chester, PA). Ethyl acetate was purchased from Fisher Scientific (Fair Lawn, NJ). Hydrochloric acid was purchased from Aqua Solutions (Deer Park, TX). Polychrom Clin II solid phase extraction columns were purchased from SPEware (Baldwin Park, CA). Cocaine hydrochloride used in hair contamination was a reference standard purchased from

United States Pharmacopeia (Rockville, MD). Drug standards for preparing calibrators were purchased from Cerilliant (Round Rock, TX).

Synthetic sweat solution was made by dissolving 1.313 g urea (EMD), 3.780 g sodium chloride (EMD), 0.384 g potassium chloride (EMD), 1.68 mL sodium lactate (ChemService, West Chester, PA), and 300 µL of Pompeian extra virgin olive oil in 1 L of DI water. Sweat solution was prepared once, just before contamination of batch 1, and divided between 3 spray bottles. One bottle was used for each batch, and the remaining bottles were stored in the refrigerator until used.

Phosphate buffer was prepared by weighing 12 gm NaH_2PO_4, (monobasic) (EMD) and 100 mg BSA (Sigma). The two reagents were dissolved in DI water and diluted to 100 mL in a volumetric flask. The pH was adjusted to 6.0 using either 10N NaOH (Fisher) or 12.1N HCl (Fisher). This buffer stock solution was stored in the refrigerator until needed for dilutions. The buffer stock solution was then further diluted 1/100, and the pH adjusted to 6 using either 10N NaOH or 12.1N HCl. Typically, 2L of the diluted buffer solution was prepared weekly. On three occasions, 4 L of the diluted buffer solution was prepared at a time for each contamination batch due to the amount of buffer needed for decontamination.

2.1.4 Hair Contamination and Decontamination Methods

The design of the experiment was adapted from Stout and colleagues (2006) and was a three-way cross-design, with subsampling. The factors investigated were time, ethnicity, and decontamination type. Specimens were removed at 11 time points with respect to contamination (i.e., pre-contamination, 1 hour, 6 hours, 24 hours, weekly) during a 56-day period (**Figure 2-1**).

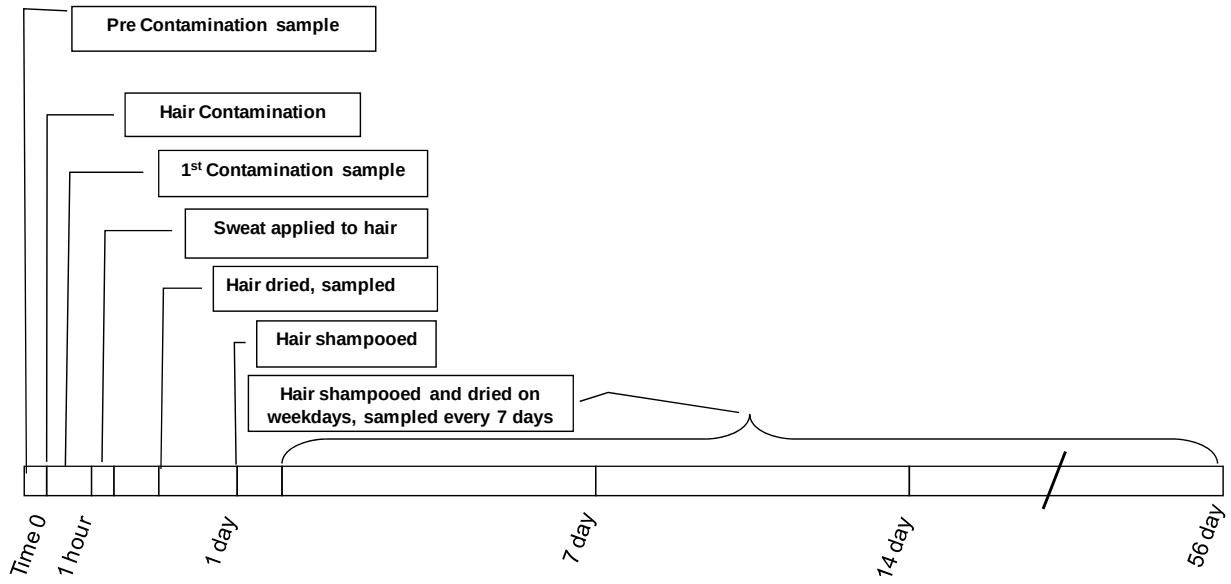


Figure 2-1. Surface contamination of hair sampling design, including contamination, sweat treatment, and sampling.

All hair types were contaminated with COC, subjected to a treatment with synthetic sweat after 1 hour, and shampooed each weekday evening (i.e., Monday through Friday) for the 56-day period. Hair was collected before contamination; after contamination, but prior to the sweat application; approximately 6 hours post-contamination (i.e., approximately 4 hours after sweat application, followed by a drying period); and then weekly for 8 weeks. Weekly specimens were collected on Monday mornings. At all points, hair was analyzed and quantitative results were obtained for cocaine analytes: COC, benzoylecgonine (BE), cocaethylene (CE), and norcocaine (NCOC).

RTI created standard protocols for the procedures used throughout the contamination study, called Batch Contamination Records (BCRs). The following is a list of the different BCRs created. An example BCR template is included in Appendix B.

1_Receipt of Donor Hair

2A_ Aliquot of donor hair for reference testing

2B_ Aliquot of donor hair for characterization studies

2C_ Aliquot of donor hair for characterization studies (post-contamination)

3_ Aliquot of donor hair for contamination study BCR

4_Preparation of Phosphate Buffer

5_Synthetic Sweat Production

6_ Stock Drug Analyte, Cocaine HCl for contamination study BCR

7_ Preparation of cocaine hydrochloride powders for Contamination study

8_Application of Coc HCl to hair sample for surface contamination study

9_Aliquoting Hair Post contamination with cocaine and decontamination of hair

9A_Aliquoting Hair Post contamination for outside analysis and microscopy

10_Analytical Preparation of Samples for LC/MS/MS Analysis

Aliquots of approximately 10 g were removed from each negative donor hair for contamination. One subject had less than 8 g of hair available for the surface contamination because less than 10 g was collected and some was lost during the washing process. Sample sizes of 10 g were desired to ensure that an adequate amount of hair remained for testing despite small amounts of hair lost in the daily washing process. The aliquots were assigned a new ID to be able to distinguish contaminated hair (Contamination ID) from the remaining negative donor hair. Batches 1 and 2 included 12 hair samples, and Batch 3 included 13 hair samples because one AA hair sample used was much smaller and was consumed before the end of the 8-week period. An additional hair sample was used so that there would still be 12 samples at the end of the 8-week period. Contamination of each batch was done on a Monday, with 2 weeks between the start of each new batch contamination. COC HCl aliquots of approximately 8 mg were weighed for each hair sample. For hair samples weighing less than 10 g, the amount of COC was adjusted so that the amount of COC relative to the amount of hair stayed consistent. Gloved hands of three

analysts were sprayed with a small amount of synthetic sweat solution and rubbed together until dry, approximately 30 seconds. The COC was distributed on the palms of the gloved hands by gently rubbing until the COC powder was no longer visible. Once the COC had been distributed on the hands, the hair was handled for 5 minutes with the intent of evenly transferring the COC to the hair. The contaminated hair was then placed between two sheets of filter paper for approximately 1 hour. Gloves were changed and the analysts repeated the process for the remaining hair samples.

Aliquots were removed from the contaminated hair at 11 time points. At each time point, three aliquots of approximately 120 mg each were removed and placed in screw-cap vials. Decontamination of time-point samples was performed immediately following sample collection. Aliquots were designated as follows: no decontamination wash process (NW), methanol decontamination (MeOH), and an extended phosphate buffer decontamination wash (PO4). The NW aliquots were collected and stored potentially for later analysis. The MeOH aliquots were performed by adding methanol to the vial at a volume to adequately cover the hair, capping the vial, and placing it on a shaker for 8 minutes. The methanol was decanted with a pipette and discarded. The same process was repeated a second time, with the methanol from the second wash being transferred to a clean, labeled vial and stored at -20°C for later analysis.

For the PO4 decontamination, isopropanol and phosphate buffer were warmed in a water bath set at 39°C. A shaker was warmed by placing a 19.7" x 11.8" IntelliTemp reptilian heating mat (Big Apple Pet Supply, NY) on the surface of the shaker. Samples were placed on top of the heating mat and covered with warmed gel packs. The hair in the vial was covered with warm isopropanol, capped, and placed on a warmed shaker for 15 minutes. The temperature was monitored several times during the first day of decontamination with an infrared thermometer to

ensure that this setup was able to keep the samples at 37–39°C throughout the decontamination process. The isopropanol was removed with a pipette and discarded. Next, the hair was covered with warm (39°C) phosphate buffer using the same vial, placed on the warmed shaker for 30 minutes, followed by removal and discard of the phosphate buffer solution. This procedure was repeated for a total of three 30-minute washes, and then two 60-minute washes. The total washing time of the extended phosphate buffer wash procedure was 3 hours and 45 minutes. At the end of the second hour wash, the buffer was transferred to a clean, labeled vial and stored at -20°C until later analysis.

The first samples (time point 1) were removed from the hair approximately 1 hour after contamination. Following removal of time point 1 samples, the hair was sprayed with synthetic sweat solution until the hair lock was saturated. The hair samples were then allowed to dry between two pieces of filter paper before the collection of time point 2 (about 4 hours). Once time point 2 samples were removed, each lock of hair was washed with warm tap water and approximately 1 mL of Pert Plus shampoo 2-in-1 medium conditioning formula for normal hair. Washed hair samples were then placed between two pieces of clean filter paper and allowed to dry overnight. Time point 3 samples were removed the next morning. The hair samples were washed each weekday afternoon and placed between two pieces of clean filter paper to dry. Time point samples 4–11 were collected each Monday for the following 8 weeks. After decontamination, samples were placed between two pieces of clean filter paper to dry. After decontamination at each time point, 10 mg of hair was weighed into a screw-cap test tube for extraction and LC-MS/MS analysis. Additionally, at time point 4, approximately 40 mg was removed from each aliquot after decontamination and sent to the reference laboratory for testing.

At time points 4, 6, 8, and 10, approximately 20 mg was removed from each sample after decontamination for microscopy analysis.

2.1.5 Analytical Sample Preparation

Calibrators were made by weighing 10 mg of drug-free hair into glass screw-cap test tubes and spiking with drug standards. Calibration curves covered the range of 25–10,000 pg/mg for COC and 2.5–4500 pg/mg for other COC analytes. Hair samples used as positive and negative controls were previously tested proficiency testing (PT) samples, NIJ reference materials, and drug user hair samples. Control hair samples were weighed into glass tubes in 10 mg aliquots. Deuterated standards were added to all tubes. The concentrations of CE-d3, BE-d3, and NCOC-d3 were 20 pg/mg, while the concentration of COC-d3 was 200 pg/mg. An extraction solution (1.5 mL) of 0.1 M potassium phosphate (pH 2.7) was added to each tube. The tubes were capped and vortexed, centrifuged at 3500 rpm for 3 minutes, and sonicated in a water bath at 65–70°C for 3 hours. Samples were cooled to ambient temperature before the buffer was decanted into clean glass tubes and 1 mL of 0.1M potassium phosphate buffer (pH 6) was added. The tubes were again vortexed and centrifuged at 3500 rpm for 3 minutes before pouring samples onto solid phase extraction (SPE) columns, which were previously conditioned with 2 mL of methanol and 1 mL of 0.1 M HCl. Samples were allowed to flow through by gravity. SPE columns were washed with the following: 2 mL each of DI water, 0.1 M HCl, methanol, and ethyl acetate with house nitrogen pressure applied for 2 minutes between each wash. Analytes were eluted with 2 mL of 78:20:2 mixture of methylene chloride:methanol:ammonium hydroxide. The elution solvent was evaporated to dryness at 40°C. Samples were reconstituted in 50 µL of methanol.

2.1.6 LC-MS/MS Analyses

A LC-MS/MS method previously used in our laboratory for the analysis of BE in urine was optimized for the detection of all four analytes by varying parameters such as mobile phase, flow rate, column type, and column temperature until the desired separation was achieved. The tandem mass spectrometry (MS/MS) method was optimized for each analyte by injection of standards and varying fragmentation and collision energies. Once the LC-MS/MS method was optimized, several extraction methods were evaluated. These methods are described briefly in the results section. The method which produced the best chromatography was employed throughout the study.

The LC-MS/MS equipment consisted of an Agilent 1200 series LC coupled to an Agilent 6410 triple-quadrupole MS with an electrospray source operating in positive mode (Santa Clara, CA). The mobile phases consisted of (A) 5 mM ammonium formate with 0.1% formic acid and (B) methanol. The mobile phase gradient is shown in **Table 2-3**.

Table 2-3. LC Gradient

Time	Flow Rate (mL/min)	% Mobile Phase B
1	0.6	5
6	0.6	40
6.1	0.6	95
8*	0.6	95

*8-min run with a 3-min post-run equilibration

All analyses used an Agilent Zorbax XDB-C18 (3.5 µm, 2.1 x 50 mm) LC column. The flow and temperature of the drying gas, which consisted of house nitrogen, were 13 L/min and 350°C, respectively. Nebulizer gas pressure was 45 psi. Collision energies and fragmentation voltages were optimized for each analyte. These parameters are shown in **Table 2-4**.

Table 2-4. MS/MS Method Parameters

Drug	Precursor Ion (m/z)	Fragmentation Voltage (V)	Collision Energy Voltage (V)	Product Ion 1 (m/z)	Collision Energy Voltage (V)	Product Ion 2 (m/z)
Cocaine	304	110	15	182	35	82
Cocaine-d3	307	120	20	185	35	85
Benzoylecgonine	290	130	15	168	25	105
Benzoylecgonine-d3	293	110	15	171	25	105
Norcocaine	290	110	20	136	25	68
Norcocaine-d3	293	110	10	171	25	136
Cocaethylene	318	130	15	196	40	82
Cocaethylene-d3	321	120	20	199	35	85

2.1.7 Analysis of Specimens by Reference Laboratory

At the 1 week time point, a specimen from each individual was submitted to Immunalysis Corporation (Pomona, CA) as an analytical reference laboratory. Specimens were weighed, packaged in glass vials, sealed in individual plastic bags, and sent by overnight carrier.

Negative control materials were prepared from each of the hair prior to COC exposure. Portions of one of the hair samples used in the study were used as negative controls several times throughout the study so that multiple determinations were made on each negative hair specimen by the laboratory. Control materials were packaged similarly to study specimens to be blind to the reference laboratories. Negative and positive control specimens were randomly inserted in each batch at a 2% frequency rate.

Positive control materials consisted of control hair preparations manufactured in association with RTI's efforts in the National Laboratory Certification Program (NLCP) Pilot Performance Testing for hair-testing laboratories conducted under contract to SAMHSA and the U.S. Department of Health and Human Services. Two different target concentrations in these control materials were used throughout the study.

2.1.8 Statistical Analysis Procedures

Data were tabulated in Microsoft Excel, and graphs were produced using Microsoft Excel. Statistical analyses were performed using SAS software. Data were log transformed prior to analysis as the data distribution was log normal.

Statistical analyses were conducted to evaluate the five outcomes of interest (i.e., COC, CE, BE, NCOC, and BE/COC). For each of these measured outcomes, RTI sought to determine the following:

1. Are there any significant differences between ethnic groups over time (crossed term of ethnicity and time)?

2. Are there any significant differences between ethnicity at all (just the ethnicity term)?

3. Are there any significant differences between decontamination treatments over time (crossed term of decontamination and time)?

4. Are there any significant differences between decontamination treatments at all (just decontamination)?

5. Are there any significant differences between ethnic groups over time by decontamination?

There were 12 (13 for African American until time point 8) individuals analyzed within each ethnicity type per timed collection point, treated as a repeated measures model with repeated measures over day. The initial linear mixed model included the effects of the following:

- Time: linear
- Time: quadratic

- Ethnicity (i.e., AA, DC, BC)
- Ethnicity by time linear
- Ethnicity by time quadratic
- Decontamination method by time linear
- Decontamination method by time quadratic.

RTI was able to determine the final estimates of the effects of time (i.e., linear, quadratic) first; then, the effects of ethnicity were determined using the Tukey test for multiple pair-wise comparisons among the three groups. The same comparison was constructed for decontamination procedures and their interactions.

2.1.9 Modifications to Research Design and Rationale

Two modifications were made to the study methods from either the originally proposed study or from previously published uses of this contamination model. First, the proposed study design wasto analyze melanin in the hair samples for a more quantitative evaluation of hair color and melanin content. Attempts of this analysis were made using a previously published method of hair digestion in tissue solubalizing agents (Solvable and Soluene) and UV-Vis spectrometry. Inconsistent results under any conditions attempted were obtained. Melanin does not exhibit any absorption maxima in spectral regions that are not related to amide or other protein interference. Thus, spectrometric measurement is not reproducible.

Second, due to criticism of the quantity of COC used in the contamination study, the amount of COC was lowered again from the proposed amount. The original study published by Stout and colleagues in 2006 used 15 mg of COC on a 10 g lock of hair. The second study (Ropero-Miller and Stout, 2009) used 8 mg of COC on an 8 g lock of hair. In this study, 8 g of

COC on a 10 g lock of hair was used. We have continued to try and reduce the quantity of COC while ensuring we can reliably handle the materials used. We were able to obtain large enough hair donations this time to be able to increase the size of the hair lock.

3. RESULTS

3.1 Evaluation of Subject Hair for Study Inclusion

Immunoassay screening for the following drug classes were included in this analysis: COC analytes (limit of detection [LOD], 50 pg/mg), phencyclidine (150 pg/mg), amphetamine/methamphetamine (250 pg/mg), tetrahydocannibinol ([THC], 50 pg/mg) and opiates (50 pg/mg). Samples that screened positive for COC were excluded from further use in the contamination study, as were hair samples that were too small to complete the study. **Table 3-1** details the results from the individuals who were excluded from this study. In all, 12 subjects were excluded from this study.

Table 3-1. Individuals Excluded from the Contamination Study

Collection ID	Accession ID	Contamination ID	Gender	Ethnicity	Study Group	Age	Type of Collection	Schwarzkopf Scale	Reason for Exclusion
071409J	11367-150-5	Excluded	Female	AA	AA	36	Cut	4	Cocaine positive
072009A	11367-150-6	Excluded	Male	AA	AA	17	Razor	3	Cocaine positive
071409H	11367-150-8	Excluded	Female	AA	AA	18	Cut	3	Cocaine positive
071409D	11367-150-11	Excluded	Male	AA	AA	30	Razor	5	Cocaine positive
071409I	11367-150-12	Excluded	Female	AA	AA	20	Cut	4	Cocaine positive
071309B	11367-150-32	Excluded	Male	Caucasian	DC	48	Razor	4	Cocaine positive
071309A	11367-150-35	Excluded	Male	Caucasian	DC	48	Razor	4	Cocaine positive
081709A	11367-150-39	Excluded	Male	AA	AA	29	Razor	1	Cocaine positive
No assignment	11367-150-40	Excluded	Male	Caucasian	BC	5	Razor	9	Drug screen negative; specimen too small (5.7 g)
012910C	11367-150-42	Excluded	Female	AA	AA	Unknown	Cut	1	Cocaine positive
012610A	11367-150-44	Excluded	Female	AA	AA	Unknown	Cut	1	Cocaine positive
071310A	11367-150-48	Excluded	Female	AA	AA	46	Cut	6	Cocaine positive

3.2 Morphological Examination

Variability both within and between individuals was quite large, and there were no obvious relationships between ethnic group and any morphological feature observed. For each individual, a composite of images from each microscopic technique was compiled (see Appendix C). For each of the composite images, the first row shows images from SEM observation of knotted hair strands. The second row contains images of SEM observation of freeze-fractured hair strands. The third and forth rows contain incidence fluorescence images of rhodamine stained hair strands and methylene blue stained hair strands. The last row contains images of stained and unstained hair strands by bright field microscopy. This composite of microscopy photos allowed for visual comparisons within and between individuals. Hair samples were highly variable between individuals with highly variable staining and highly variable cuticle appearance. Incidence fluorescence did allow for the visualization of staining patterns on opaque hair strands for both methylene blue and rhodamine. The hair strands that exhibited the very smooth surface appearance, which were also the hair samples with the very homogenous staining appearance, on freeze fracturing appeared to have either a very thin, non-scaly cuticle or lacked cuticle scales (**Figure 3-1**). From the freeze-fracture images, the number of cuticle scales was counted.

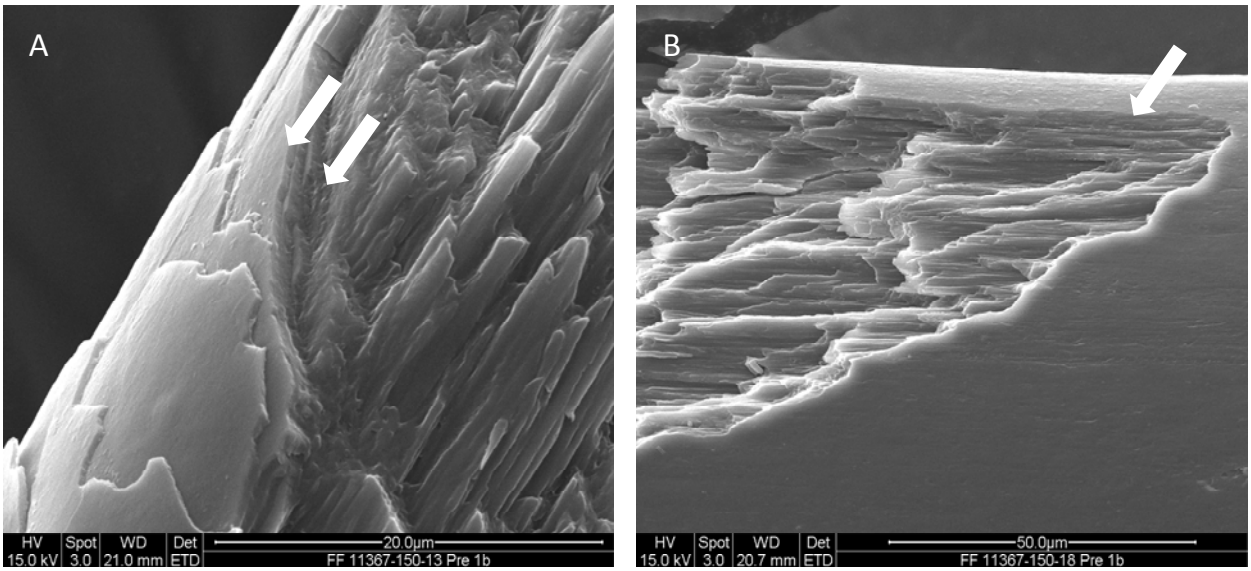


Figure 3-1. A comparison of freeze fracture images from a BC female with obvious cuticle scales (A) and a BC female with no obvious cuticle scales.

Note the obvious cuticle layers in (A, arrows) and the either very thin or minimal layers in (B, arrow).

The average number of cuticle scale layers from each of the ethnic groupings displayed by decontamination treatment with pretreated hair, post methanolic decontamination, and post extended phosphate buffer decontamination is presented in **Figure 3-2**. The error bars represent 1 standard deviation for the mean, and each bar represents the mean of all of the individuals. There were no significant differences in the number of cuticular layers between either the ethnic groupings or the decontamination treatment groups.

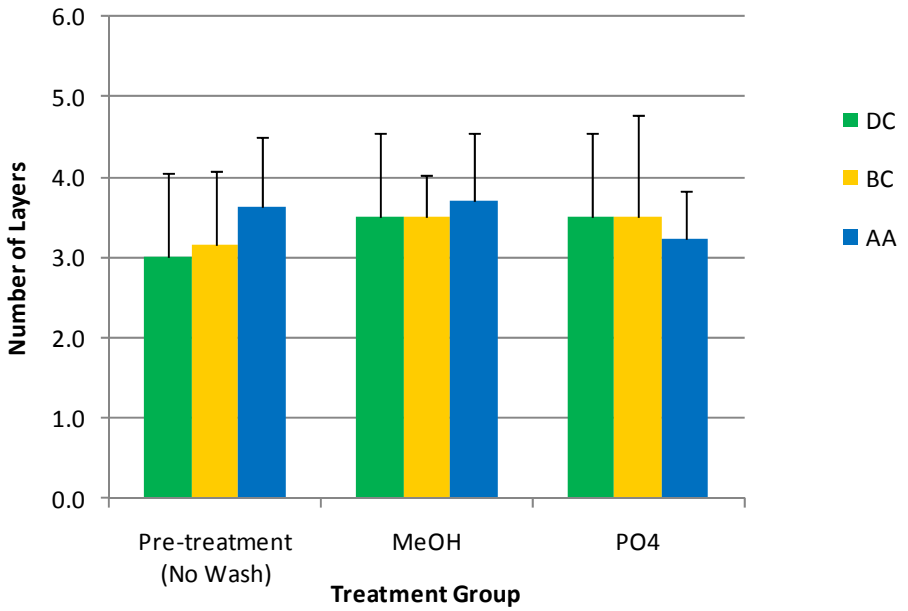


Figure 3-2. Average number of cuticular layers observed by ethnicity and by decontamination treatment.

When the average thickness of the cuticle layer was examined, the overall cuticular thickness found for all individuals was typically between 2 and 5 micrometers. Between-individual variation was quite large, and there were no apparent significant differences in thickness between either ethnic groupings or decontamination treatments (**Figure 3-3**)

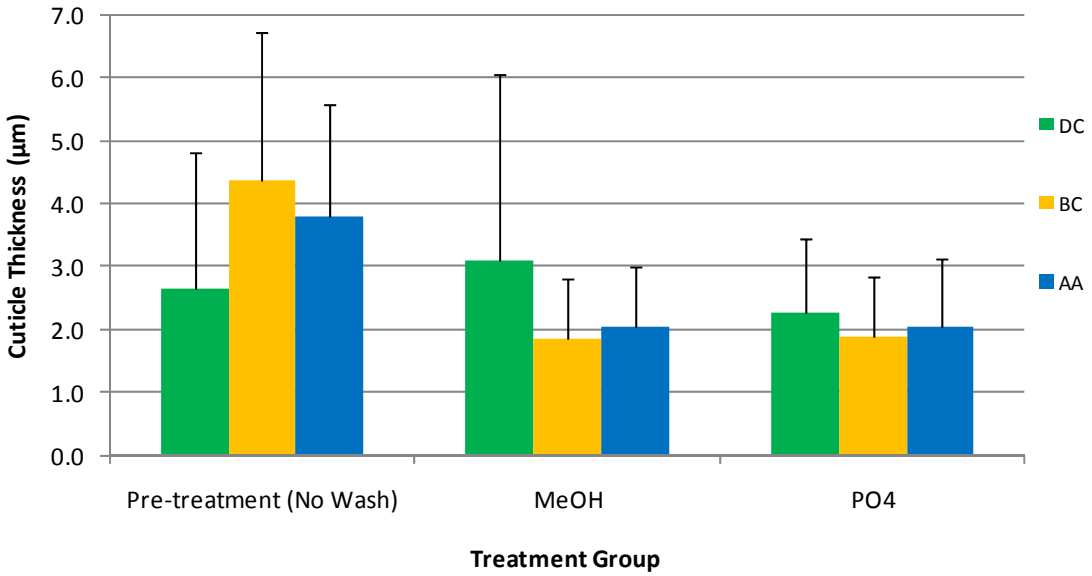


Figure 3-3. Cuticle thickness in µM by ethnic group and by decontamination treatment.

Significant differences in the penetration of staining were observed between hair samples of the same ethnicity and hair samples of different ethnicities, both prior to and after decontamination treatment. The extent of stain penetration can be associated with the amount of damage to the hair, such as separation of or missing cuticle scales. Many of the hair samples showed damage by decontamination demonstrated by a greater extent of stain penetration or by signs of fractures. SEM was used to examine the appearance of cuticle scales, cuticle thickness, and number of cuticular layers. Comparisons of images taken after the phosphate buffer decontamination with pre-contamination images show damage to most of the hair samples, including lifted and broken cuticle scales, fractures, and abrasion of the hair surface (**Figure 3-4**). This damage could be caused by hydration of the hair fibers with warm buffer, and friction between hair fibers, as they were vigorously shaken for several hours. Many of the hair samples looked similar before and after methanol decontamination. Some of the hair samples, however, do show lifting of cuticle scales after methanol treatment, but there was no consistent pattern to this appearance.

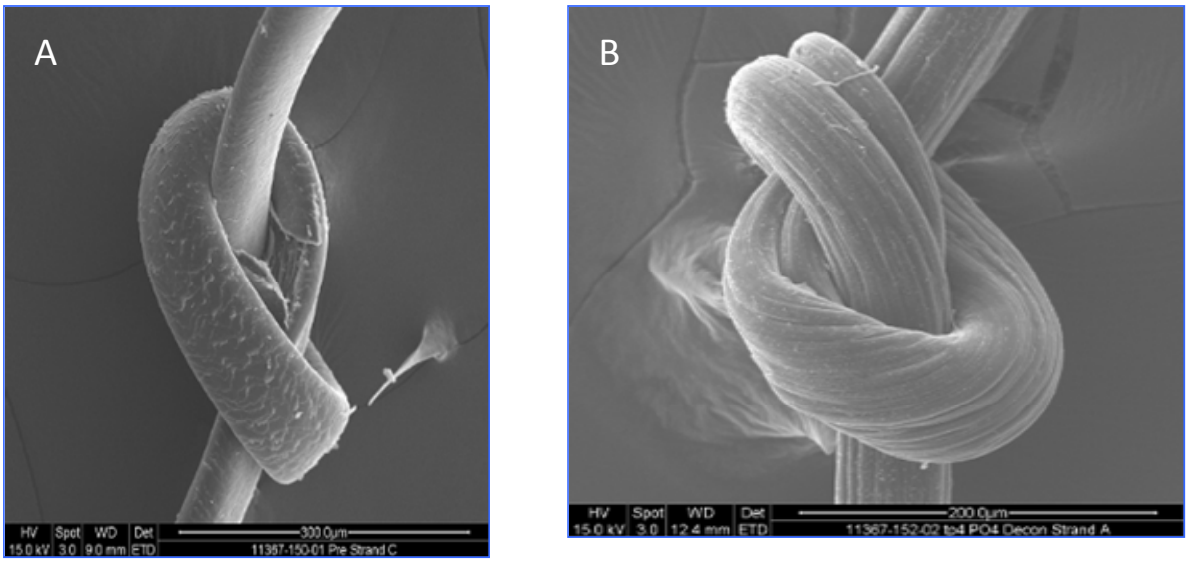


Figure 3-4. A comparison of knotted region of a male AA sample before (A) and after (B) phosphate decontamination.

This example demonstrates very obvious differences. Other individuals had much less marked change (see Appendix C for a full suite of images)

Images of the hair samples were evaluated to determine if there was any correlation between COC concentration and hair damage. The images were first separated into two categories, one containing hair samples resulting in high COC concentrations, and the other comprised of hair samples resulting in low concentrations. Each category was reviewed to determine if there was any correlation between the degree of hair damage and the amount of COC concentration. A second way of dividing the hair samples was to select the hair samples with the smoothest appearance from the SEM images and then look for trends in the analyte concentrations found. Next, the images were separated into two categories based on the degree of methylene blue staining to determine if the amount of staining correlated with COC concentration. After examination, it was determined that there was no correlation between hair damage and concentration.

Lastly, from the results of the application of the decision criteria, those hair samples that had multiple positive calls throughout the study period were examined for any common appearances. Again, no consistent patterns were observed. Moreover, the group of individuals that were called "positive" had both hair strands that stained very heavily and individuals with minimal staining (**Figures 3-5 and 3-6**). Figure 3-5 shows the composite images from individual 11367-150-30 (DC) which had a total of 16 "positive" analyses across the time period after both methanol and phosphate decontamination (with only one positive eliminated by the wash criterion) using decision criteria 1.

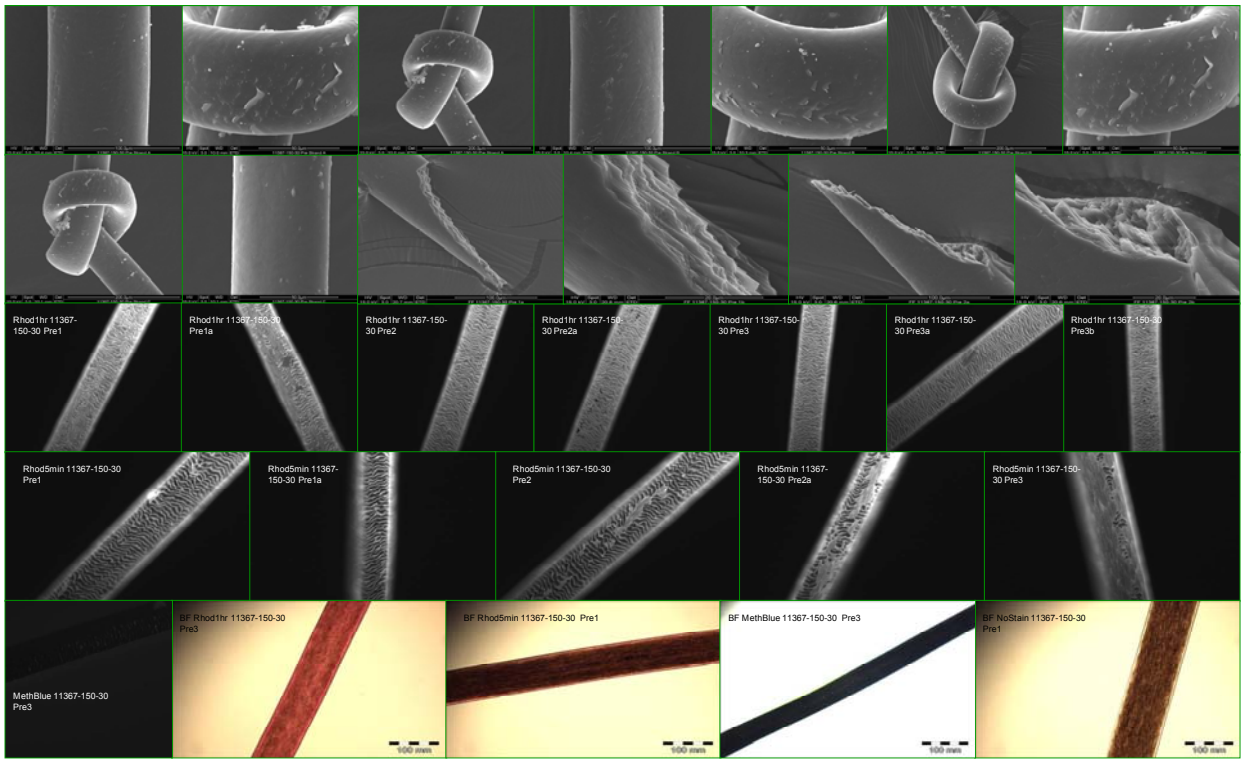


Figure 3-5. Hair donor [11367-150-30 (DC)] with strong rhodamine staining, minimal methylene blue staining, and higher concentrations of COC (16 of 22 time point analyses determined to be a positive result by decision Criteria 1).

Note the strong staining by rhodamine, largely only visible in the fluorescence microscopy due to the very dark color, and the minimal staining by methylene blue, again in the fluorescence microscopy. This is in comparison to the composite image of individual 11367-150-29 (DC) (**Figure 3-6**), which had a total of 7 "positive" analyses across the time period after both phosphate and methanol decontamination (with 5 positives eliminated by the application of the wash criterion).

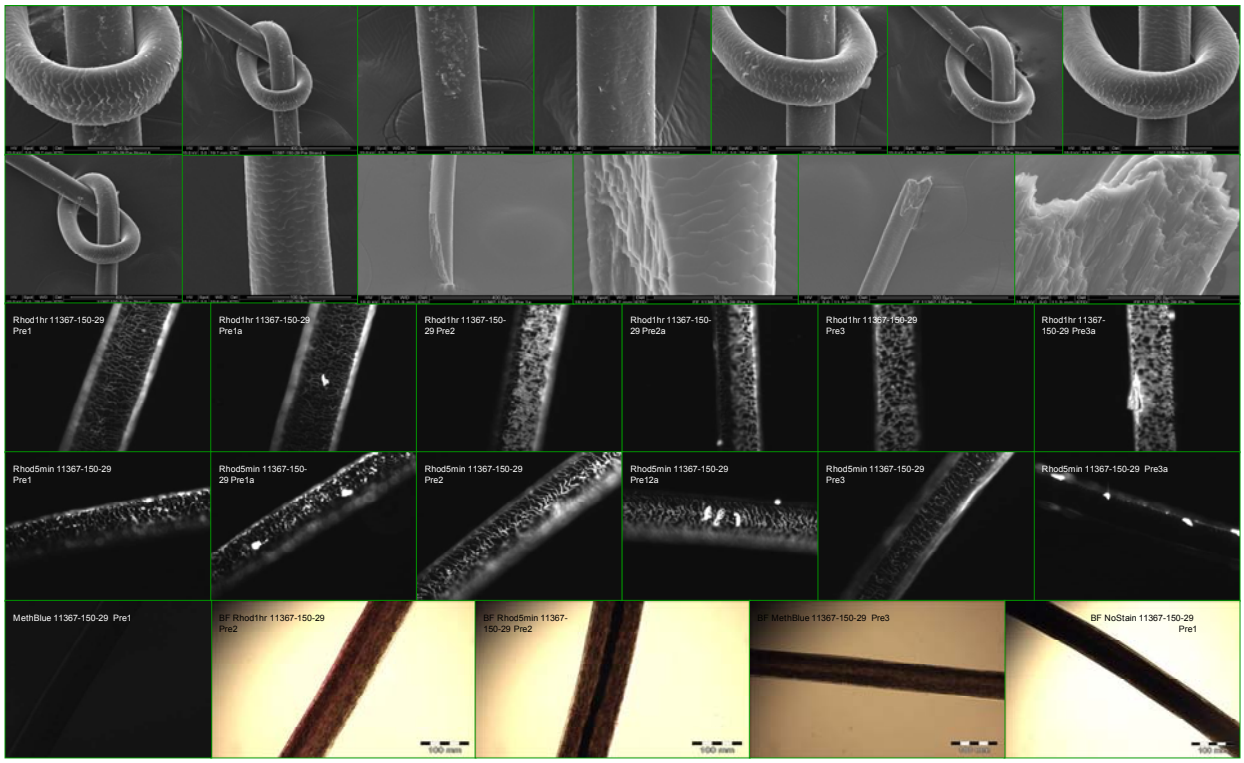


Figure 3-6. Hair donor [11367-150-29 (DC)] with minimal methylene blue or rhodamine staining and lower concentrations of COC (7 of 22 time point analyses determined to be a positive result by decision criteria 1).

Note the comparatively minimal staining either by methylene blue or rhodamine, and the unremarkable cuticle appearance in SEM. Both of these individuals are compared to individual 11367-150-19 (BC) (**Figure 3-7**), which demonstrated strong staining by both methylene blue and rhodamine and also strongly staining damage structures, but only had positive calls after methanol decontamination and only for CE criteria and no positives after phosphate buffer decontamination.

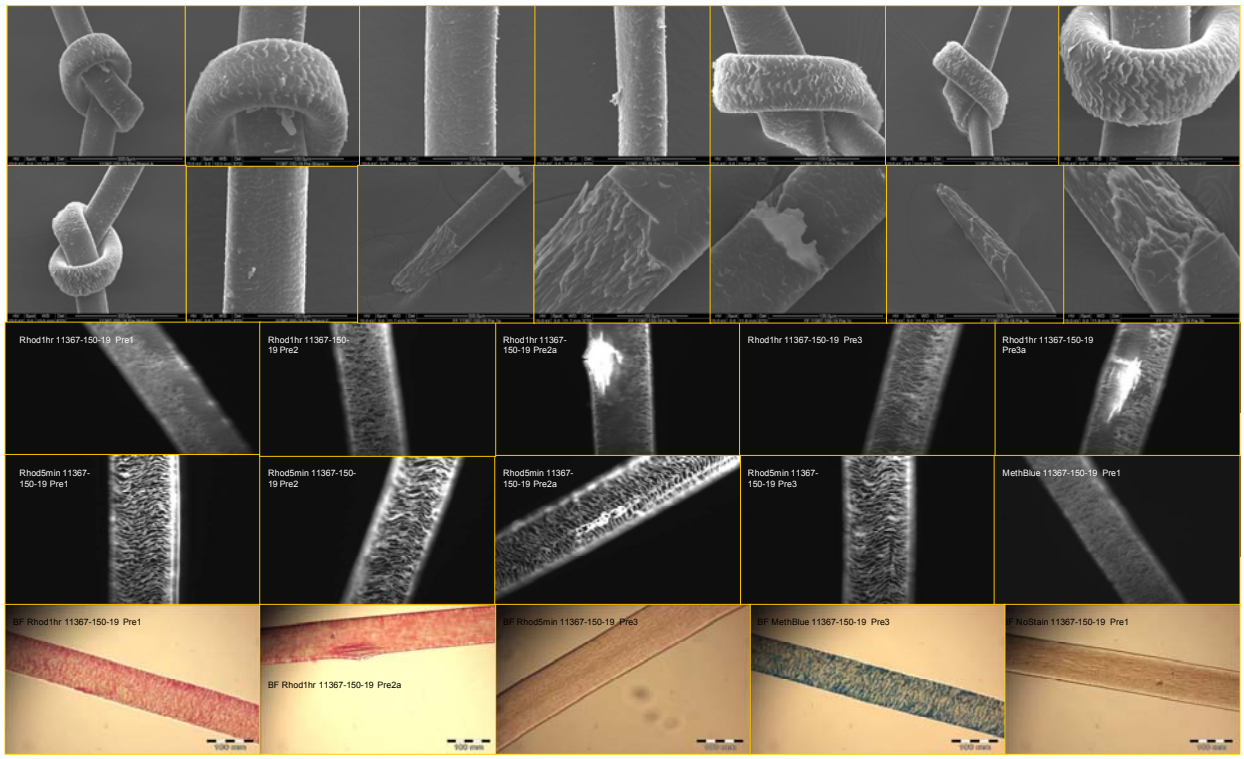


Figure 3-7. Hair donor [11367-150-19 (BC)] with strong rhodamine and methylene blue staining and low concentrations of COC (0 of 22 time point analysis determined to be a positive result by decision Criteria 1).

Images of methylene blue staining obtained in this study exhibited a markedly different appearance than the images in Hill and colleagues (2004). Likely, this is due to the use of a higher-quality microscope that allowed for better resolution of the cuticle scale pattern of staining. Every effort was made to duplicate the staining method published by Hill and others, but the diffuse appearance of staining evident in Hill and colleagues was not apparent in our study.

3.3 Validation of COC, BE, CE, and NCOC

Prior to validation, the hair-extraction method was adjusted to determine which parameters produced the best chromatography and optimum response and sensitivity. The method previously described was performed with the following incubation parameters: overnight at pH 2.7, 3 hours at pH 2.7, and overnight in a hair digest solution made of dithiothreitol (DTT)

and Protease K at pH 7.4. After evaluation, it was determined that an incubation time of 3 hours at pH 2.7 produced the most favorable results, with sensitivity ratios of 5:1 or better.

Linearity, precision, and accuracy (LPA) samples were prepared and analyzed at RTI using LC-MS/MS. Past National Laboratory Certification Program (NLCP) proficiency, reference, and drug-user material were used as controls. The concentrations for the LPA samples are described in **Table 3-2**.

Table 3-2. Detail of the Distribution of Sample Concentrations in the Linearity, Precision and Accuracy (LPA) Samples

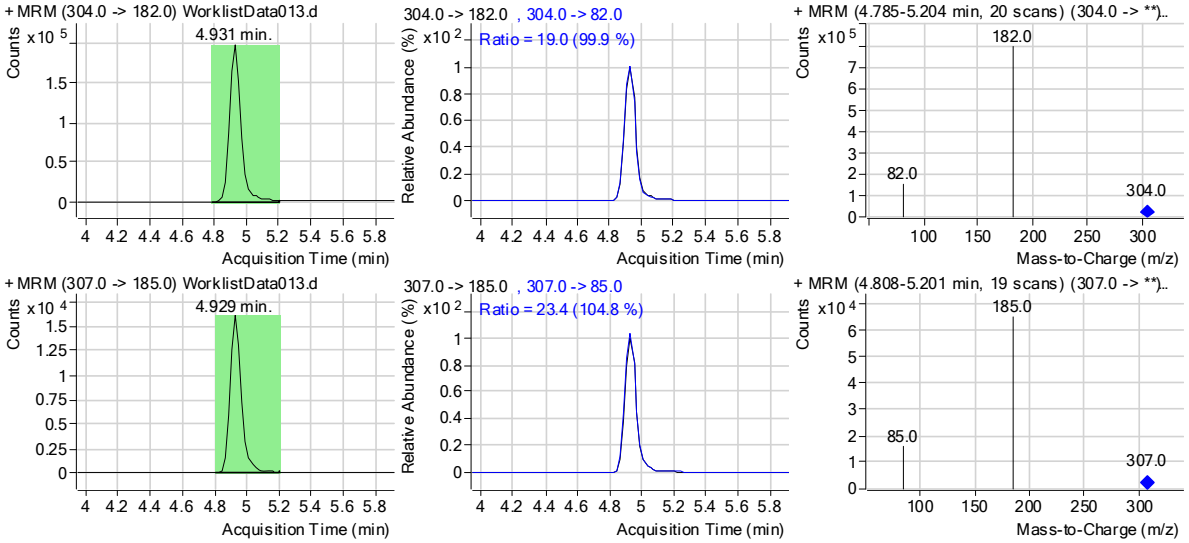
Sample Set	COC(pg/mg)	BE(pg/mg)	CE(pg/mg)	Norcocaine(pg/mg)
1	50	5	5	5
2	150	15	15	15
3	250	25	25	25
4	500	50	50	50
5	2000	200	200	200
6	8000	800	800	800

Each set of samples was extracted and analyzed 10 times using 5 separate calibrations within a 5-day period. **Table 3-3** and **Figure 3-8** show validation statistics and representative LC-MS/MS chromatography and spectra for the hair extraction method used in this study.

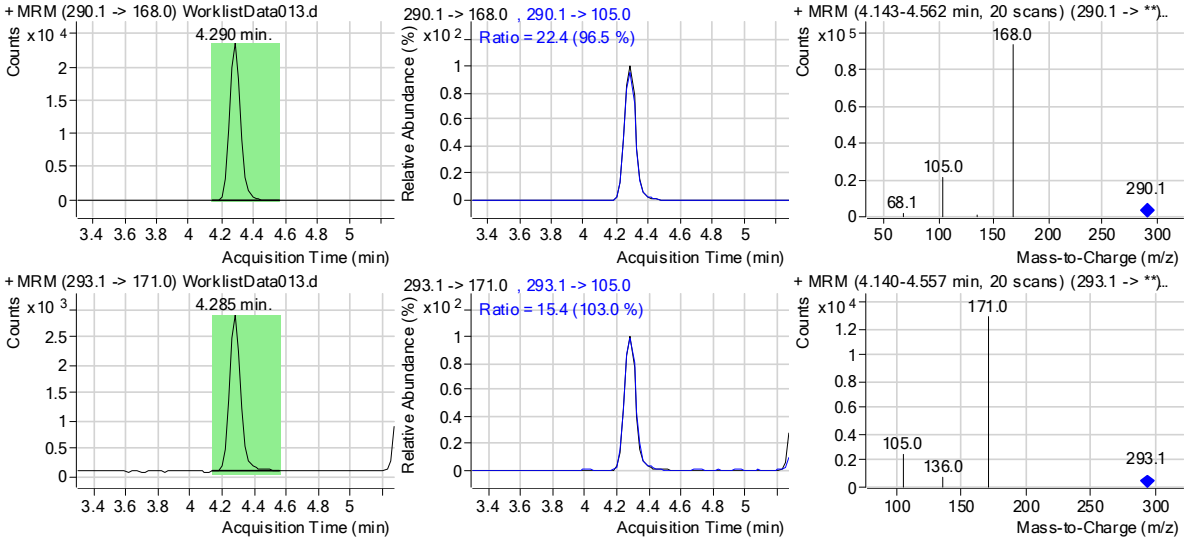
Table 3-3. Validation Statistics for the Linearity, Precision and Accuracy (LPA) Study

Drug	n	Limit of Detection (pg/mg)	Limit of Quantitation (pg/mg)	Average %CV	Average Accuracy	Average Accuracy %CV
COC	60	25	25	3.97	100.83	7.72
BE	60	2.5	2.5	7.15	105.50	7.19
CE	60	2.5	2.5	6.15	100.53	8.68
Norcocaine	60	2.5	2.5	6.23	100.49	8.45

COC



BE



(continued)

Figure 3-8. Typical chromatography and monitored MRM for each target compound (top row) and its respective internal standard (bottom row) from the LC-MS-MS analysis.

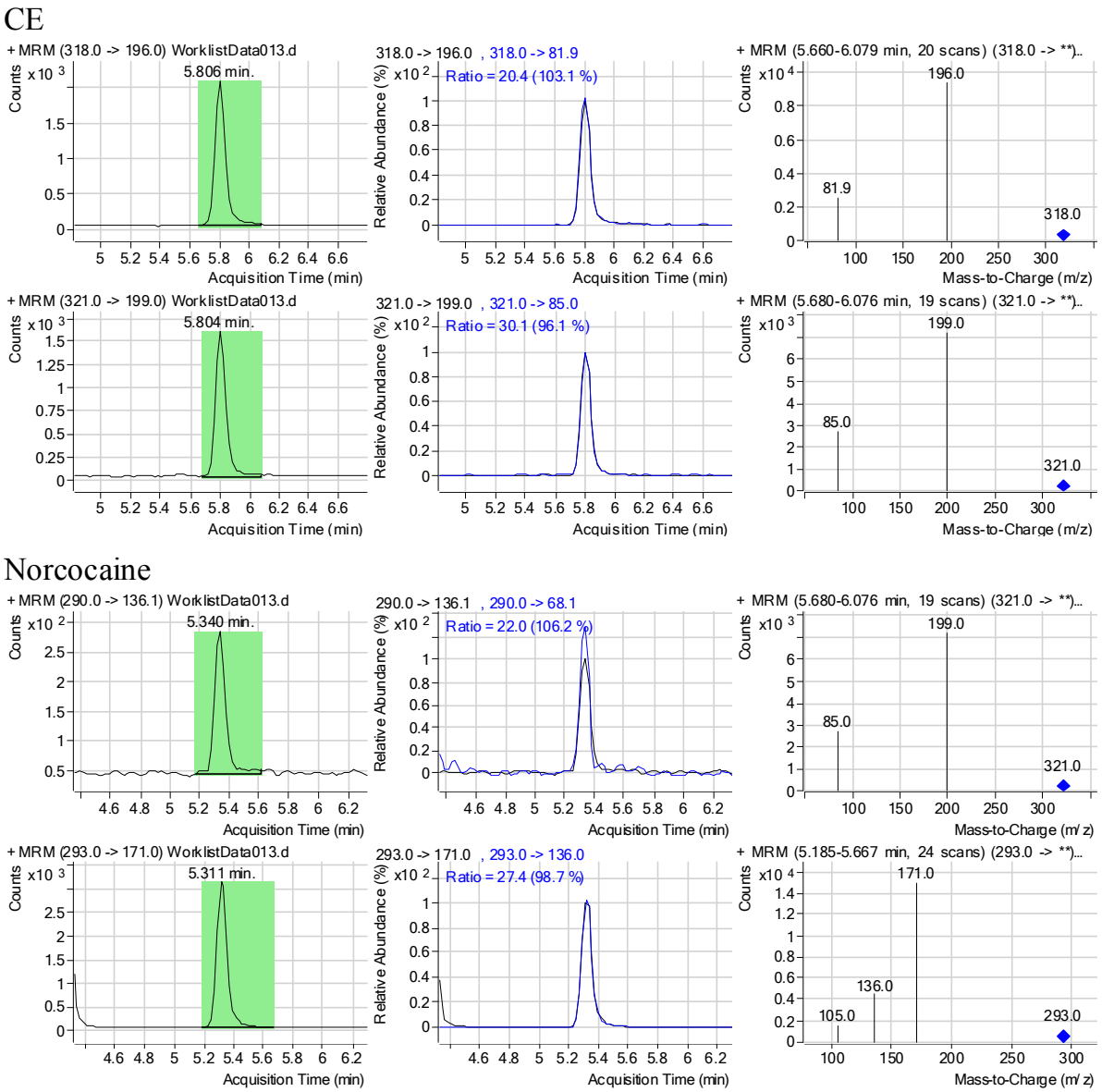


Figure 3-8. Typical chromatography and monitored MRM for each target compound (top row) and its respective internal standard (bottom row) from the LC-MS-MS analysis (continued).

The LOD and limit of quantitation (LOQ) were set at the lowest point on the calibration curve. The average %CV for the drug concentrations over the 10 analyses and their accuracies was less than 7.2% and 8.7%, respectively.

Matrix effect samples contained 200 pg/mg of COC and 20 pg/mL of each metabolite. For the purpose of this study, 10 lots of hair, each from a different donor, were collected, spiked, and analyzed once. Matrix effects were evaluated using the methods described by Matuszewski

and colleagues (2003). Three sets of samples were created for each target analyte. Type A, or neat, samples were prepared by spiking target analyte and internal standard in mobile phase. Type B, or post-extraction, samples were prepared by spiking target analyte and internal standard into the eluent of the solid phase extracted hair matrix. Finally, Type C, or pre-extraction, samples were prepared by spiking target analyte and internal standards into the negative hair matrix prior to solid phase extraction. Comparative calculations were used to evaluate the matrix effect (ME), recovery effect (RE), and process efficiency (PE):

$$ME\ (\%) = B/A \times 100$$

$$RE\ (\%) = C/B \times 100$$

$$PE\ (\%) = C/A \times 100$$

where

A, B, and C = the mean responses as represented by the area under the peaks for target and internal standard quantitative transitions.

Table 3-4 shows the results of the ME study.

Table 3-4. Matrix Effect Study Results

Drug	Target Ion Response			ISTD Ion Response			%CV		
	ME (%)	RE (%)	PE (%)	ME (%)	RE (%)	PE (%)	Type A	Type B	Type C
COC	156.19	75.89	118.53	148.83	74.73	111.23	7.45	3.69	5.66
BE	113.57	77.80	88.35	100.81	76.67	77.28	4.54	12.89	19.99
CE	143.06	68.59	98.12	139.87	71.55	100.08	5.65	5.53	4.76
NCOC	168.12	72.31	121.56	163.45	76.68	125.33	7.03	4.21	5.54

Although the ME values are greater than 100%, the %CV for the responses are less than 13%, with the exception of Type C samples for BE, which has a %CV of 19.99%. This is due to the use of matrix-matched calibrators and controls and stable isotope-labeled internal standards.

3.4 Analyte Concentrations after Decontamination

3.4.1 Cocaine

For COC (**Figure 3-9**), there were significant effects of time on COC concentration levels (i.e., linear and/or quadratic components [$p<0.0001$]). This is consistent with prior studies RTI has conducted in the pattern and concentrations of COC after surface contamination with COC HCl powder. AA hair had significantly greater concentrations of COC than BC ($p=0.0004$) or DC ($p=0.0464$) groups when the hair was decontaminated with the methanolic decontamination. AA hair also had significantly greater concentrations of COC than BC ($p=0.156$) individuals after the phosphate decontamination. The groups with the steepest declines were the groups with the highest maximum COC levels over time (see Figure 3-9). The phosphate decontamination produced COC concentrations significantly lower than the methanolic decontamination ($p<0.0001$).

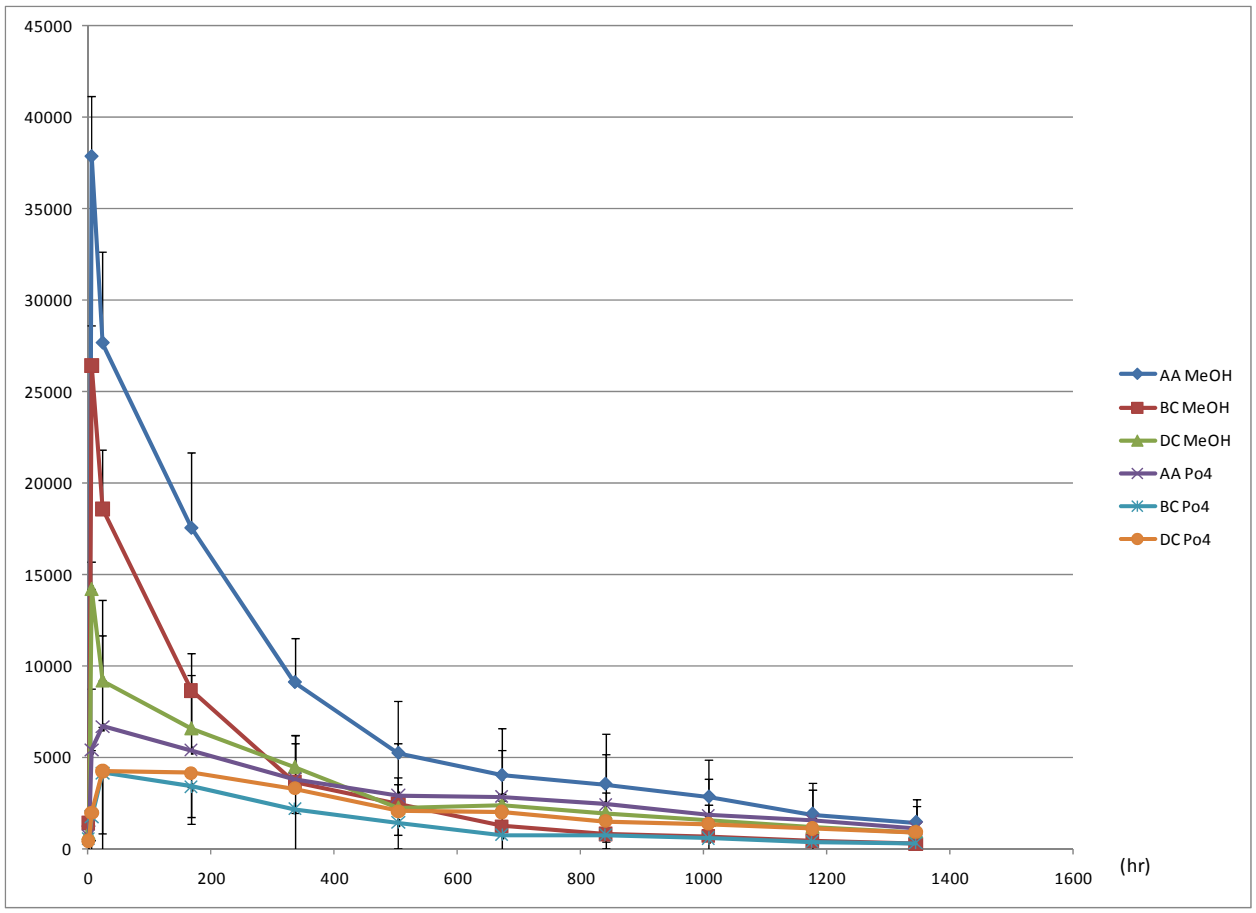


Figure 3-9. Average COC (pg/mg) for each ethnic group and decontamination over the study period (hours).

For all of the figures, the points represent the mean, and the error bars represent plus or minus 1 standard deviation. As the error bars are large due to the large between-individual variation, **Table 3-5** is provides the COC results by time point, with mean, median, and concentration range results for comparison.

Table 3-5. Characteristics of Cocaine Concentration Data by Ethnic Group

COC Concentration (pg/mg)	DC	BC	AA	DC	BC	AA
	1 hour (TP 1) MeOH			1 hour (TP 1) PO4		
Min	94	129	157	20	26	40
Max	2186	5319	2534	2415	1790	1808
Mean	813	1407	1191	427	545	686
Median	661	727	1021	200	340	404

COC Concentration (pg/mg)	DC	BC	AA	DC	BC	AA
	6 hours (TP 2) MeOH			**6 hours (TP 2) PO4**		
Min	2102	8047	7362	154	371	846
Max	80804	53211	81518	8171	5691	10813
Mean	14182	26425	37831	1972	1709	5407
Median	8980	23402	34141	1744	1139	5024
	24 hours (TP 3) MeOH			**24 hours (TP 3) PO4**		
Min	2683	5015	2646	728	676	1333
Max	20713	44623	88490	10695	16224	16297
Mean	9173	18583	27638	4232	4128	6677
Median	9058	17736	24596	2965	1935	5002
	168 hours (TP 4) MeOH			**168 hours (TP 4) PO4**		
Min	1964	2639	3523	1231	995	770
Max	13398	16700	42088	7185	9528	13599
Mean	6548	8641	17509	4123	3393	5367
Median	5402	8258	16800	3723	2842	3655
	336 hours (TP 5) MeOH			**336 hours (TP 5) PO4**		
Min	935	596	2705	1066	785	335
Max	10755	8299	24494	10244	5470	7823
Mean	4437	3645	9075	3289	2177	3793
Median	7977	2507	8845	2774	1897	3617
	504 hours (TP 6) MeOH			**504 hours (TP 6) PO4**		
Min	927.5248	467	1007	723	371	183
Max	6600.134	7725	11968	6089	4856	9414
Mean	2209.418	2468	5199	2040	1420	2898
Median	1590.771	1548	4533	1575	906	1070
	672 hours (TP 7) MeOH			**672 hours (TP 7) PO4**		
Min	639	313	1047	266	228	103
Max	6350	4053	9830	5688	2433	8233
Mean	2390	1211	3995	2006	752	2840
Median	2104	763	3514	1772	469	1524
	840 hours (TP 8) MeOH			**840 hours (TP 8) PO4**		
Min	393	204	639	566	97	151
Max	4704	4221	9257	3367	3977	7430
Mean	1935	811	3512	1506	741	2409
Median	1736	474	1955	1393	269	747
	1008 hours (TP 9) MeOH			**1008 hours (TP 9) PO4**		
Min	680	178	284	488	101	73
Max	3481	3106	8972	2837	3123	5204
Mean	1530	678	2816	1335	563	1827
Median	1287	355	1871	1086	269	662

COC Concentration (pg/mg)	DC	BC	AA	DC	BC	AA
	1176 hours (TP 10) MeOH			1176 hours (TP 10) PO4		
Min	363	74	226	520	48	94
Max	2255	2105	6472	2366	1509	6087
Mean	1161	433	1879	1093	367	1519
Median	895	207	967	817	161	988
	1344 hours (TP 11) MeOH			1344 hours (TP 11) PO4		
Min	246	17	153	282	8	43
Max	1790	1279	3674	1825	1941	3883
Mean	901	243	1429	895	296	1101
Median	642	83	709	654	71	588

3.4.2 CE

For CE (**Figure 3-10**), there were significant effects of time on CE concentration levels as with COC (i.e., linear and/or quadratic components [$p<0.0001$]). Again these results are consistent with prior studies RTI has conducted in the pattern and concentrations of CE after surface contamination with COC. AA hair had significantly greater concentrations of CE than BC ($p=0.0.0054$) individuals when the hair was decontaminated with the methanolic decontamination. AA hair had significantly greater concentrations of CE than BC ($p=0.0193$) individuals after the phosphate decontamination. The phosphate decontamination produced CE concentrations significantly lower than the methanolic decontamination ($p<0.0001$). The groups with the steepest declines were also the groups with the highest maximum CE levels over time (Figure 3-10). **Table 3-6** shows the mean, median and range of CE concentration for each ethnic group and hair collection time point.

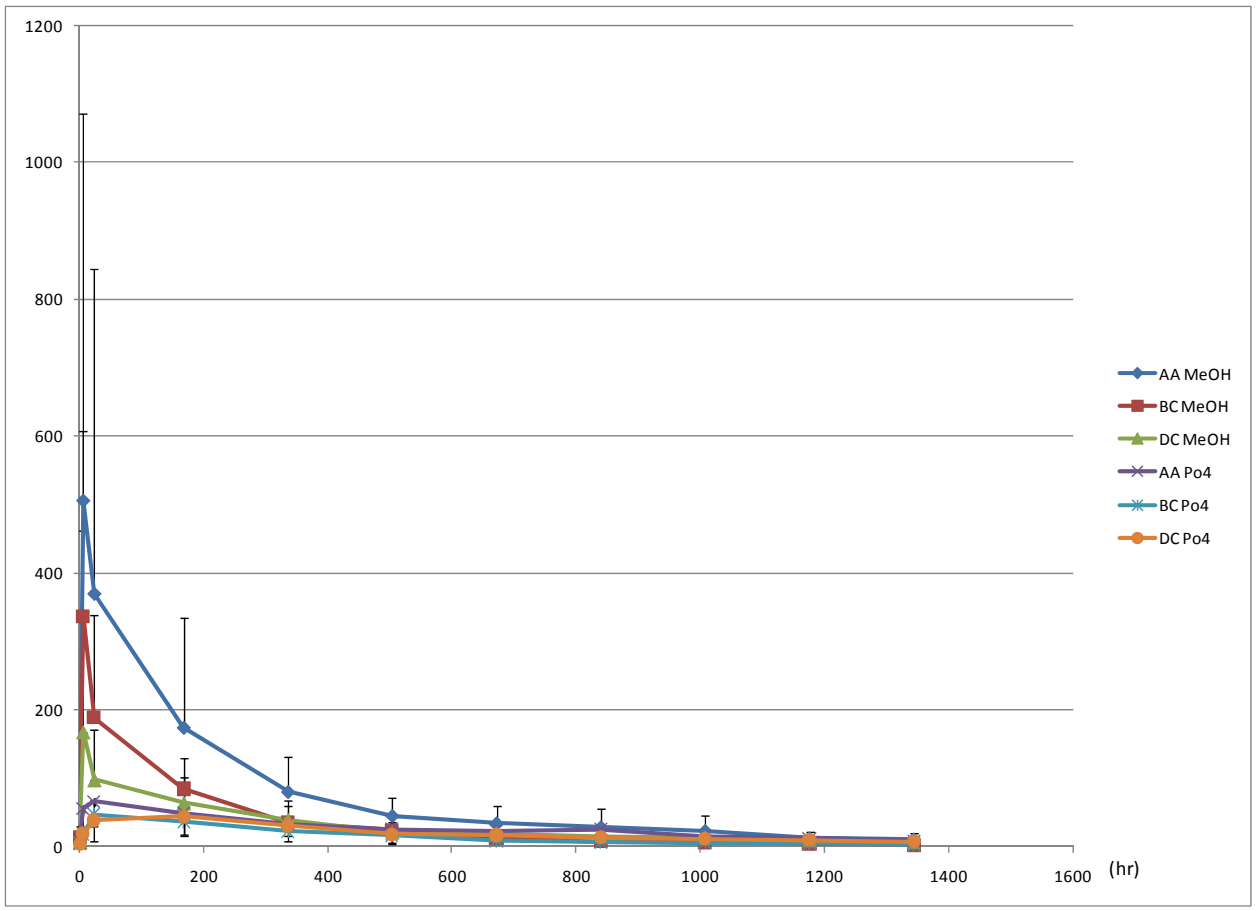


Figure 3-10. Average CE (pg/mg) for each ethnic group and decontamination over the study period (hours).

Table 3-6. Characteristics of CE Concentration Data by Ethnic Group

CE concentration (pg/mg)	DC	BC	AA	DC	BC	AA
	1 hour (TP 1) MeOH			1 hour (TP 1) PO4		
Min	2	0	1	0	1	0
Max	54	37	20	16	18	23
Mean	9	10	7	9	4	7
Median	4	7	5	9	2	4
	6 hours (TP 2) MeOH			6 hours (TP 2) PO4		
Min	30	6	14	2	7	2
Max	243	890	1093	677	2082	1144
Mean	102	281	203	115	221	194
Median	81	87	72	66	29	22
	24 hours (TP 3) MeOH			6 hours (TP 3) PO4		
Min	24	15	13	6	14	7
Max	551	441	276	321	1412	1400
Mean	131	126	116	96	165	184
Median	87	71	110	46	46	54

CE concentration (pg/mg)	DC	BC	AA	DC	BC	AA
	168 hours (TP 4) MeOH			**168 hours (TP 4) PO4**		
Min	17	33	12	10	9	15
Max	143	323	165	189	473	479
Mean	64	83	59	66	87	97
Median	52	52	50	39	49	49
	336 hours (TP 5) MeOH			**336 hours (TP 5) PO4**		
Min	6	6	8	9	9	9
Max	90	152	86	77	132	197
Mean	41	43	34	35	38	51
Median	42	26	28	36	26	39
	504 hours (TP 6) MeOH			**504 hours (TP 6) PO4**		
Min	4	5	8	4	6	5
Max	72	95	79	54	53	81
Mean	27	27	24	20	21	30
Median	19	18	13	16	18	16
	672 hours (TP 7) MeOH			**672 hours (TP 7) PO4**		
Min	1	2	4	4	2	1
Max	46	104	61	47	37	48
Mean	22	23	18	18	15	20
Median	19	12	16	14	13	17
	840 hours (TP 8) MeOH			**840 hours (TP 8) PO4**		
Min	0	0	1	1	1	2
Max	51	93	61	53	31	48
Mean	18	21	13	19	10	14
Median	13	10	8	9	7	9
	1008 hours (TP 9) MeOH			**1008 hours (TP 9) PO4**		
Min	1	0	2	1	2	2
Max	37	59	42	34	31	73
Mean	16	11	11	15	8	14
Median	12	6	5	9	5	10
	1176 hours (TP 10) MeOH			**1176 hours (TP 10) PO4**		
Min	1	1	0	1	0	0
Max	29	34	26	28	26	18
Mean	11	7	8	10	6	8
Median	9	4	5	7	4	7
	1344 hours (TP 11) MeOH			**1344 hours (TP 11) PO4**		
Min	0	0	0	1	0	0
Max	22	27	25	27	13	16
Mean	8	5	7	9	4	6
Median	5	2	5	4	2	4

3.4.3 NCOC

For NCOC (**Figure 3-10**), there were significant effects of time on NCOC levels, as with COC (i.e., linear and/or quadratic components [p<0.0001]). Again, these results are consistent with prior studies we have conducted in the pattern and concentrations of COC after contamination. AA individuals had significantly greater concentrations of NCOC than BC individuals after methanolic decontamination (p=0.0096). The phosphate decontamination produced NCOC concentrations significantly lower than the methanolic decontamination (p<0.0001). The groups with the steepest declines were also the groups with the highest maximum NCOC levels over time (**Figure 3-11**). **Table 3-7** shows the mean, median, and range of NCOC concentration for each ethnic group and hair collection time point.

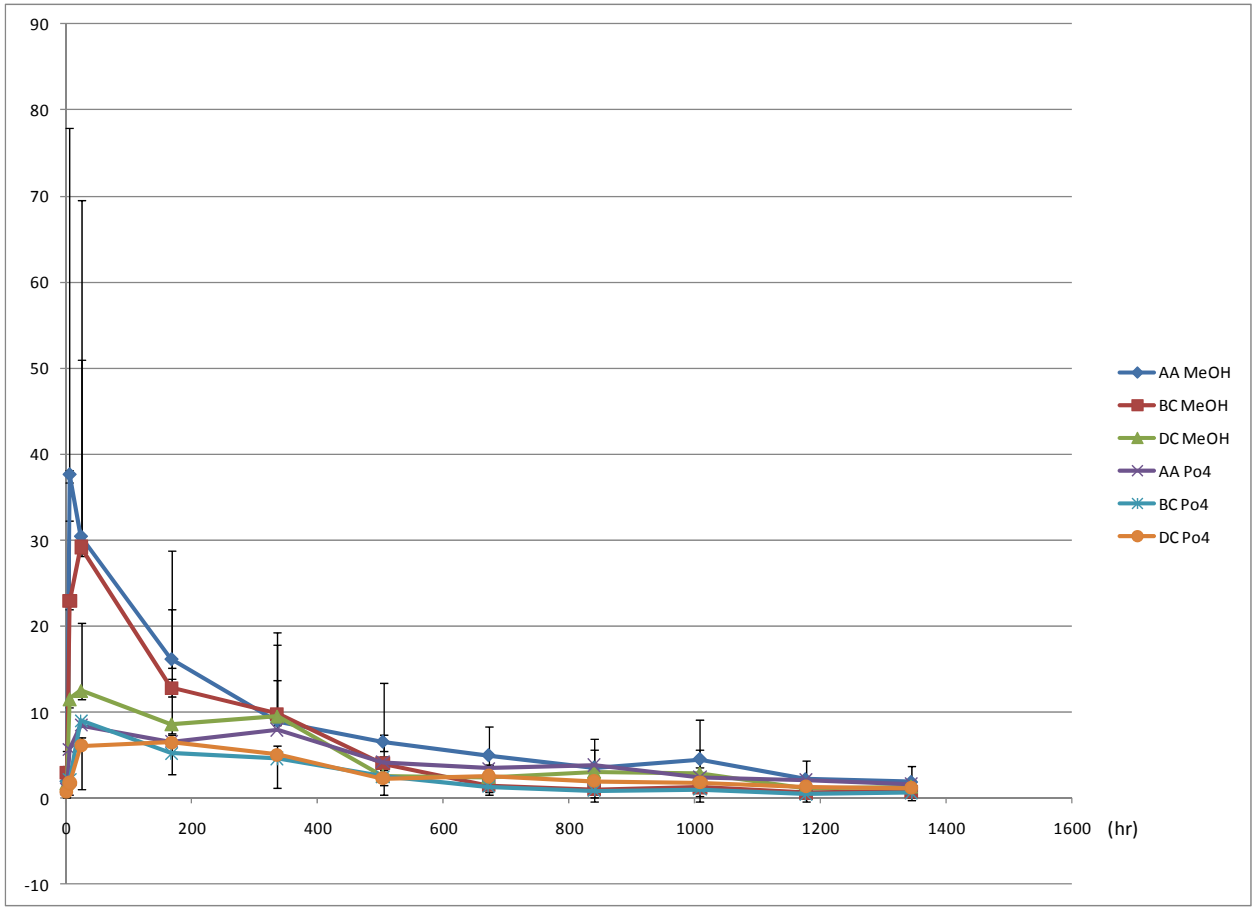


Figure 3-11. Average NCOC (pg/mg) for ethnic group and decontaminations over the study period (hours).

Table 3-7. Characteristics of Norcocaine Concentration Data by Ethnic Group

NCOC Concentration (pg/mg)	DC	BC	AA	DC	BC	AA
	1 hour (TP 1) MeOH			1 hour (TP 1) PO4		
Min	0	0	0	0	0	0
Max	9	6	4	6	2	4
Mean	2	2	1	2	1	1
Median	1	2	1	2	1	1
	6 hours (TP 2) MeOH			6 hours (TP 2) PO4		
Min	2	1	1	0	1	0
Max	31	63	77	36	113	129
Mean	10	18	15	8	13	18
Median	7	7	7	5	3	3
	24 hours (TP 3) MeOH			24 hours (TP 3) PO4		
Min	3	4	2	0	2	2
Max	75	46	53	32	78	139
Mean	19	18	17	10	12	19
Median	10	15	10	6	6	8
	168 hours (TP 4) MeOH			168 hours (TP 4) PO4		
Min	2	3	1	1	2	2
Max	18	22	37	16	38	43
Mean	9	9	11	8	9	10
Median	8	7	6	9	5	6
	336 hours (TP 5) MeOH			336 hours (TP 5) PO4		
Min	1	0	0	0	0	1
Max	15	27	33	21	15	21
Mean	6	9	10	7	7	6
Median	5	8	6	5	6	4
	504 hours (TP 6) MeOH			504 hours (TP 6) PO4		
Min	0	1	0	0	0	0
Max	12	8	19	8	6	27
Mean	4	3	4	3	3	5
Median	3	2	4	3	2	3
	672 hours (TP 7) MeOH			672 hours (TP 7) PO4		
Min	0	0	0	1	1	0
Max	11	11	8	5	4	9
Mean	4	2	2	3	2	3
Median	3	2	1	3	2	3
	840 hours (TP 8) MeOH			840 hours (TP 8) PO4		
Min	0	0	0	0	0	0
Max	11	10	10	8	5	5
Mean	4	3	2	3	2	1
Median	3	2	1	2	1	1

NCOC Concentration (pg/mg)	DC	BC	AA	DC	BC	AA
	1008 hours (TP 9) MeOH			1008 hours (TP 9) PO4		
Min	0	0	0	0	0	0
Max	11	6	6	10	3	15
Mean	4	2	2	3	1	2
Median	4	1	2	2	1	1
	1176 hours (TP 10) MeOH			1176 hours (TP 10) PO4		
Min	0	0	0	0	0	0
Max	10	7	3	5	2	2
Mean	2	1	1	2	1	1
Median	1	0	1	1	0	1
	1344 hours (TP 11) MeOH			1344 hours (TP 11) PO4		
Min	0	0	0	0	0	0
Max	4	4	6	4	3	3
Mean	2	1	1	2	1	1
Median	2	0	1	1	1	1

3.4.4 BE

For BE (**Figure 3-12**), there were significant effects of time on BE concentration levels as with COC (i.e., linear and/or quadratic components [$p<0.0001$]). Again, these results are consistent with prior studies RTI has conducted in the pattern and concentrations of CE after surface contamination with COC. No significant differences in BE concentration were observed between ethnic groups for either decontamination. The phosphate decontamination produced BE concentrations significantly lower than the methanolic decontamination ($p<0.0001$). The groups with the steepest declines were also the groups with the highest maximum BE concentrations over time (Figure 3-12). **Table 3-8** shows the mean, median, and range of BE concentration for each ethnic group and hair collection time point.

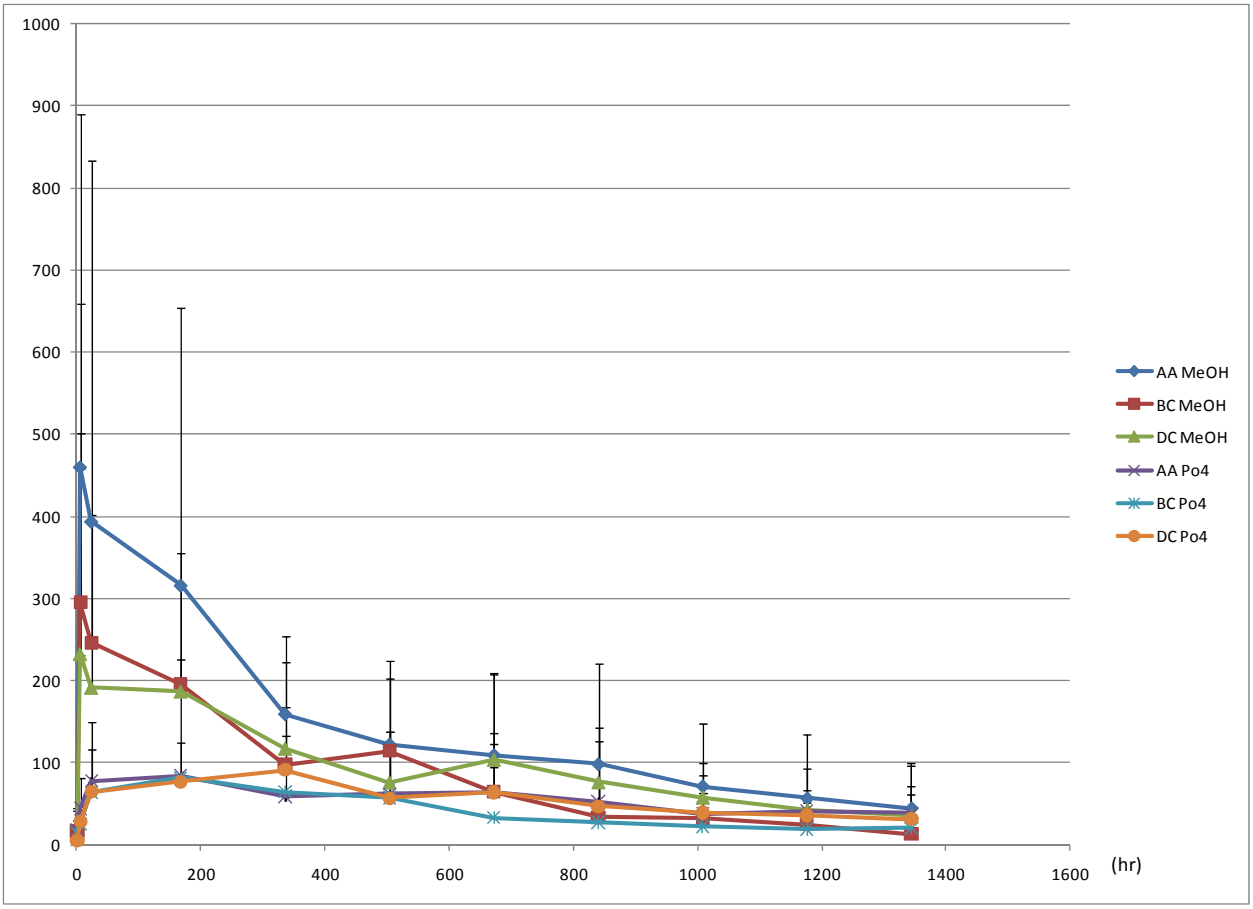


Figure 3-12. Average BE (pg/mg) for each ethnic group and decontamination over the study period (hours).

Table 3-8. Characteristics of BE Concentration Data by Ethnic Group

BE Concentration (pg/mg)	DC	BC	AA	DC	BC	AA
	1 hour (TP 1) MeOH			1 hour (TP 1) PO4		
Min	1	0	1	0	0	0
Max	103	84	28	20	59	39
Mean	21	19	11	11	11	13
Median	13	11	9	12	4	12
	6 hours (TP 2) MeOH			6 hours (TP 2) PO4		
Min	6	13	16	3	10	1
Max	221	708	1567	472	1663	854
Mean	114	234	231	117	189	204
Median	134	151	63	56	37	32
	24 hours (TP 3) MeOH			24 hours (TP 3) PO4		
Min	4	19	17	5	15	6
Max	537	426	398	671	1492	1159
Mean	192	158	158	153	184	201

BE Concentration (pg/mg)	DC	BC	AA	DC	BC	AA
Median	153	120	123	99	45	74
	168 hours (TP 4) MeOH			**168 hours (TP 4) PO4**		
Min	6	57	11	4	31	18
Max	326	306	393	624	916	1107
Mean	140	143	122	187	152	202
Median	121	96	95	118	77	81
	336 hours (TP 5) MeOH			**336 hours (TP 5) PO4**		
Min	1	16	8	14	25	13
Max	342	370	203	260	196	371
Mean	121	108	84	83	75	119
Median	129	84	71	49	68	73
	504 hours (TP 6) MeOH			**504 hours (TP 6) PO4**		
Min	2	20	7	9	22	14
Max	390	156	222	228	106	270
Mean	113	72	80	78	50	96
Median	58	50	44	48	35	53
	672 hours (TP 7) MeOH			**672 hours (TP 7) PO4**		
Min	1	14	5	11	8	6
Max	367	169	182	370	107	227
Mean	114	72	63	85	41	68
Median	74	35	34	38	32	47
	840 hours (TP 8) MeOH			**840 hours (TP 8) PO4**		
Min	0	5	3	5	8	7
Max	384	166	186	263	76	183
Mean	85	52	43	83	28	44
Median	45	24	24	29	18	31
	1008 hours (TP 9) MeOH			**1008 hours (TP 9) PO4**		
Min	2	1	3	6	4	9
Max	164	98	113	176	57	246
Mean	60	34	38	59	22	47
Median	40	23	23	27	15	33
	1176 hours (TP 10) MeOH			**1176 hours (TP 10) PO4**		
Min	0	2	3	8	4	2
Max	170	54	76	269	50	68
Mean	60	22	32	59	18	29
Median	52	21	19	32	14	21
	1344 hours (TP 11) MeOH			**1344 hours (TP 11) PO4**		
Min	2	0	0	1	0	0
Max	126	56	138	202	48	67
Mean	47	18	29	54	14	21
Median	30	8	20	22	8	19

3.4.5 BE/COC Ratios

For the BE/COC ratio (**Figure 3-13**), the linear and quadratic trends over time were significant, as has been previously observed (both analyses, $p<0.0001$). As previously reported, the BE/COC ratio increased over the study period for all groups and treatments. AA individuals had significantly lower BE/COC ratios than did BC individuals ($p<0.0001$), and significantly lower BE/COC ratio than DC individuals ($p=0.0153$). There was no significant difference in BE/COC ratios between the methanol-decontaminated hair and the phosphate buffer-decontaminated hair. This suggests several possibilities: BZE is more strongly retained than COC under all conditions tested, BZE is differentially extracted due to the pH of the extraction method, and BZE is likely generating in situ from COC deposited on the hair due to contamination. The significantly greater ratios observed in the BC group suggests these mechanisms are more prevalent in these individuals than in the AA group, even though the target drug concentration was significantly greater in the AA group than the BC group.

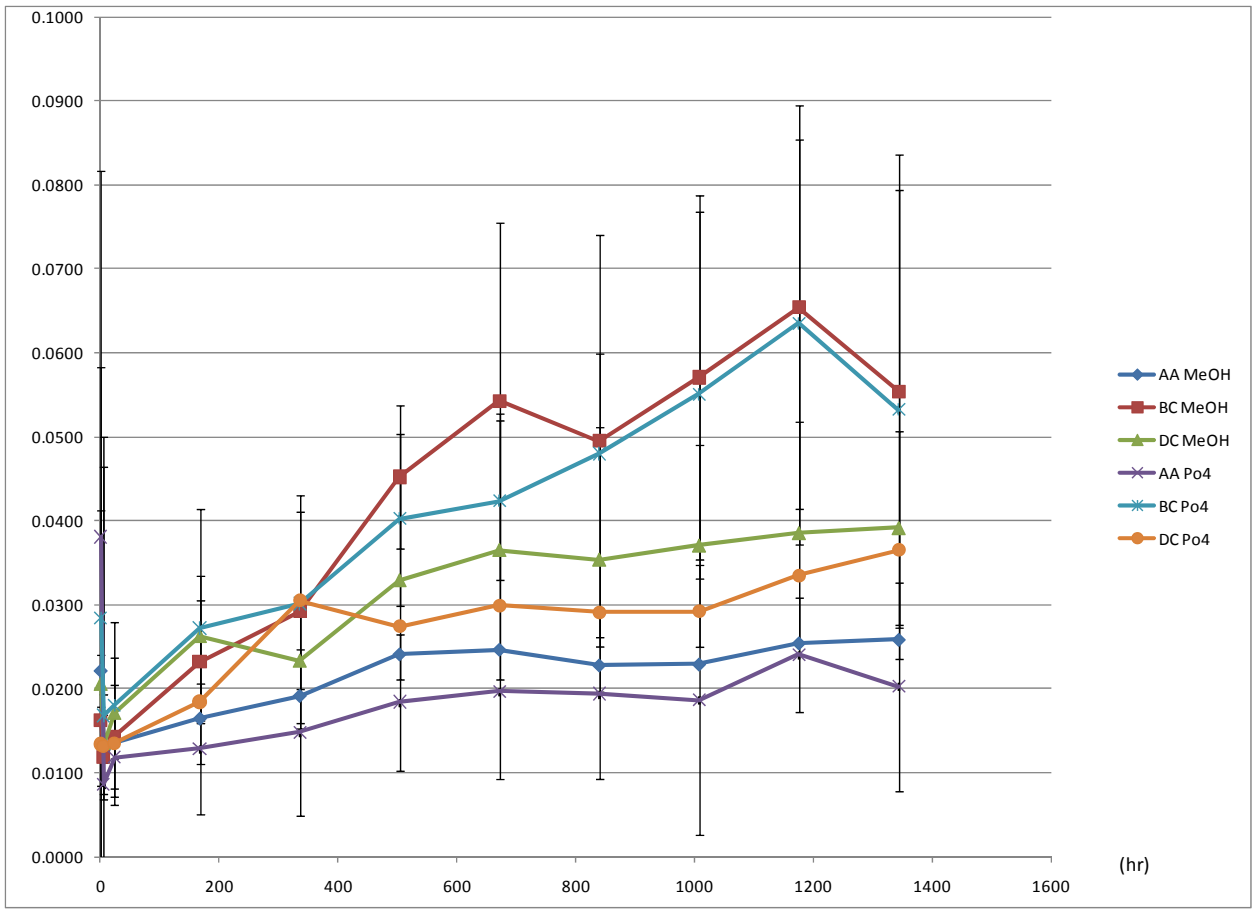


Figure 3-13. Average BE/COC ratio over the study period (hours) for each ethnic group and decontamination.

3.4.6 Decision Criteria

The analytical results also were compared against several decision criteria, as were the other hair specimens in the study. These criteria are listed in **Table 3-9**. The first three criteria are listed in this table because they are the criteria in the proposed federal regulations. Four analytes are routinely investigated in COC hair testing: COC, BE, CE, and NCOC. Typically COC is the most abundant analyte, followed by BE (5–50% COC concentration), CE (<20% COC), and NCOC (<10% COC) (Ropero-Miller at al., 2009). Reported studies focused on COC and BE after contaminating hair with COC because these are the primary analytes in hair and CE and NCOC were believed to be *in vivo* COC metabolites, not manufacturing by-products. More recent studies (Ropero-Miller and Stout, 2009; Scheidweiler et al., 2005), demonstrated that

these analytes can occur at appreciable concentrations following high-purity COC HCl subcutaneous administration, and after short COC exposure to hair followed by 10 weeks of daily hygienic treatment (i.e., shampooing). Additional decision points were selected based on review of published hair concentration data and previously proposed criteria (Stout et al., 2006; Bourland et al., 2000; Schaffer et al., 2007). Because CE and NCOC to COC ratios may be useful, Criteria 4 and 5 evaluated NCOC/COC ratios of ≥0.05 and ≥0.01, respectively, and Criteria 6 through 9 evaluated CE/COC ratios of ≥0.05, ≥0.02, ≥0.01, and ≥0.002, respectively.

Table 3-9. Criteria Used for Decision Points of Positive Calls on Analytical Results

Cocaine Analyte Concentrations and Ratios Criteria for the Determination of Confirmation Results as Positive or Negative	
Criteria 1 (BE criteria)	COC ≥500 pg/mg and BE ≥50 pg/mg and BE/COC ≥0.05
Criteria 2 (CE criteria)	COC ≥500 pg/mg and CE ≥50
Criteria 3 (NCOC criteria)	COC ≥500 pg/mg and NCOC ≥50
Criteria 4	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.05
Criteria 5	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.01
Criteria 6	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.05
Criteria 7	COC ≥ 500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.02
Criteria 8	COC ≥ 500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.01
Criteria 9	COC ≥ 500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.002

Table 3-10 summarizes the counts and relative percentages of analyses that would result in a positive call by the given criteria for each individual within an ethnic group. In Table 3-10. the results are reported per individual. Positive results were counted by individual over the study period, and then a percentage calculated in comparison to the number of individuals in that group. Alternatively, all analyses were treated separately in **Table 3-11**, so the percentages are relative to the total number of analyses conducted for that treatment group, including replicate analyses, that is all hair tests performed.

Table 3-10. Comparison of COC Contaminated Hair Test Results to Decision Criteria for Individuals

Criteria for a Positive COC Hair Test		Number (%) of COC Positive Individuals from Contaminated Hair Populations Based on each Criteria					
		African American n = 13		Blonde Caucasian n = 12		Dark Caucasian n = 12	
		Methanol Decon.	PO_4 Decon.	Methanol Decon.	PO_4 Decon.	Methanol Decon.	PO_4 Decon.
Criteria 1 (BE criteria)	COC ≥500 pg/mg and BE ≥50 pg/mg and BE/COC ≥0.05	3 (23)	6 (46)	8 (67)	7 (58)	9 (75)	6 (50)
Criteria 2 (CE criteria)	COC ≥500 pg/mg and CE ≥50 pg/mg	10 (77)	7 (54)	10 (83)	9 (75)	10 (83)	8 (67)
Criteria 3 (NCOC criteria)	COC ≥500 pg/mg and NCOC ≥50 pg/mg	2 (15)	1 (8)	2 (17)	1 (8)	2 (17)	0
Criteria 4	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.05	0	1 (8)	0	0	0	0
Criteria 5	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.01	0	1 (8)	0	1 (8)	0	0
Criteria 6	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.05	1 (8)	2 (15)	2 (17)	5 (42)	0	5 (42)
Criteria 7	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.02	4 (31)	4 (31)	5 (42)	8 (67)	5 (42)	6 (50)
Criteria 8	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.01	4 (31)	6 (46)	7 (58)	9 (75)	10 (83)	8 (67)
Criteria 9	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.002	9 (69)	7 (54)	9 (75)	9 (75)	10 (83)	8 (67)

Table 3-11. Comparison of COC Contaminated Hair Test Results to Decision Criteria for Differentiating COC Use from COC HCl Contamination

Criteria for a Positive COC Hair Test		Number (%) of COC Positive Hair Tests from Contaminated Hair Populations Based on each Criteria					
		African American n = 139		Blonde Caucasian n = 132		Dark Caucasian n = 132	
		Methanol Decon.	PO_4 Decon.	Methanol Decon.	PO_4 Decon.	Methanol Decon.	PO_4 Decon.
Criteria 1 (BE criteria)	COC ≥500 pg/mg and BE ≥50 pg/mg and BE/COC ≥0.05	8 (6)	15 (11)	16 (12)	17 (13)	26	30
Criteria 2 (CE criteria)	COC ≥500 pg/mg and CE ≥50 pg/mg	31 (22)	23 (17)	29 (22)	21 (16)	35	19
Criteria 3 (NCOC criteria)	COC ≥500 pg/mg and NCOC ≥50 pg/mg	2 (1)	2 (1)	2 (2)	2 (2)	2 (2)	2 (2)
Criteria 4	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.05	0	2 (1)	0	0	0	0
Criteria 5	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.01	0	2 (1)	0	1 (1)	0	0
Criteria 6	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.05	1 (1)	7 (5)	6 (5)	9 (7)	0	10 (8)
Criteria 7	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.02	7 (5)	12 (9)	11 (8)	19 (14)	11 (8)	14 (11)
Criteria 8	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.01	9 (6)	19 (14)	16 (12)	21 (16)	25 (19)	18 (14)
Criteria 9	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.002	27 (19)	23 (17)	26 (20)	21 (16)	34 (26)	19 (14)

3.4.7 Addition of Wash Criterion

In addition to the decision criteria applied in Section 3.4.6, the wash criteria, as described by Cairns and colleagues (2004b), was also applied to the hair COC results. In addition to the decision criteria applied in Section 3.4.6, the wash criteria, as described by Cairns and colleagues (2004b), was also applied to the hair COC results. The application of a "wash criterion" decision is calculated by multiplying 5 times the COC concentration of the last wash solution in the

extended phosphate buffer decontamination and subtracting this from the hair COC concentration ($COC_{Wash\ Criterion} = COC_{Original\ hair\ concentration} - 5*COC_{Last\ PO4\ wash\ concentration}$). If this calculated result still exceeds the decision criteria, it is a "positive" call. Decontamination washes were collected and analyzed at time points 1, 3, and 11 to evaluate the addition of a laboratory wash criterion.

Notable differences in "positive" calls were observed after the application of the wash criteria. At time point one, only one positive call would have been made by Criteria 1 in a BC individual and 1 positive by the CE criteria in a DC individual. These two individuals were not positive after the application of the wash criteria.

At time point 3, positive calls prior to the application of the wash criteria and after the wash criteria are detailed in **Table 3-12**. All positive calls related to BE criteria were eliminated by the application of the wash criteria, but five individuals had positive calls by several CE criteria after the application of the wash criteria. Notably, all of these were AA individuals.

Table 3-12. Comparison of COC Contaminated Hair Test Results to Decision Criteria for Individuals Before and After the Application of the "Wash Criterion"

Criteria for a Positive COC Hair Test		Number ([before wash criterion] / [after wash criterion]) of COC Positive Individuals from Contaminated Hair Populations Based on each decision Criteria					
		African American n = 13		Blonde Caucasian n = 12		Dark Caucasian n = 12	
		Methanol Decon.	PO_4 Decon.	Methanol Decon.	PO_4 Decon.	Methanol Decon.	PO_4 Decon.
Criteria 1 (BE criteria)	COC ≥500 pg/mg and BE ≥50 pg/mg and BE/COC ≥0.05	1/0	2/0	1/0	4/0	0	4/0
Criteria 2 (CE criteria)	COC ≥500 pg/mg and CE ≥50 pg/mg	10/2	7/3	8/0	6/0	10/0	5/0
Criteria 3 (NCOC criteria)	COC ≥500 pg/mg and NCOC ≥50 pg/mg	1/0	1/0	0	1/0	2/0	0

Criteria for a Positive COC Hair Test		Number ([before wash criterion] / [after wash criterion]) of COC Positive Individuals from Contaminated Hair Populations Based on each decision Criteria					
		African American n = 13		Blonde Caucasian n = 12		Dark Caucasian n = 12	
		Methanol Decon.	PO_4 Decon.	Methanol Decon.	PO_4 Decon.	Methanol Decon.	PO_4 Decon.
Criteria 4	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.05	0	1/0	0	0	0	0
Criteria 5	COC ≥500 pg/mg and NCOC ≥50 pg/mg and NCOC/COC ≥0.01	0	1/0	0	0	0	0
Criteria 6	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.05	0	2/0	1/0	3/0	0	2/0
Criteria 7	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.02	2/0	3/1	2/0	6/0	3/0	3/0
Criteria 8	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.01	3/0	6/2	4/0	6/0	6/0	5/0
Criteria 9	COC ≥500 pg/mg and CE ≥50 pg/mg and CE/COC ≥0.002	9/2	7/3	8/0	6/0	10/0	5/0

At time point 11, eight individuals had positive calls by Criteria 1 before the application of the wash criteria, and seven individuals had positive calls after the wash criteria. The one AA individual who was positive by this criterion at this time point was eliminated as positive by the wash criteria.

The wash criteria appeared to have the greatest impact at early time points. At later time points, the ratio of BE/COC is larger in some individuals, and the COC present appears to be more resistant to removal. The wash criterion is less effective in these hair samples. Hence, with the use of wash criterion using an extended phosphate buffer decontamination, there does not appear to be an ethnic relationship.

3.4.8 Parallel Results Study (RTI and Reference Laboratory)

To evaluate if testing performed at RTI using a newly validated LC/MS/MS procedure was acceptable, a specimen from each individual was submitted to Immunalysis Corporation (Pomona, CA) as an analytical reference laboratory. An aliquot at one week time point was collected for a total of 74 samples analyzed for comparison. Both samples decontaminated with methanol and phosphate buffer were included in the parallel study. COC concentrations in these samples demonstrated an average absolute percent difference of 26%, and RTI values were consistently lower. Similar results were demonstrated CE, with an average absolute percent difference of 31%, but the reference laboratory did not detect NCOC, as all samples were less than their LOQ of 50 pg/mg. BE results had the highest variation in results from RTI and Immunalysis, demonstrating an average absolute percent difference of 26%. In 73 of 74 samples, RTI had lower BE concentrations than Immunalysis. Based on inter-laboratory precision of hair drug proficiency testing results reported in the literature, it was concluded that RTI LC-MS-MS methodology consistently performed (Ropero-Miller et al., 2007b; Ventura et al., 2007; Society of Hair Testing, 2009).

3.5 Conclusions

Environmental exposure of drugs continues to be a concern for hair drug testing studies, child protection services, probation and parole, and the criminal justice system. It is essential to the validity of hair drug testing that a drug user can be easily identified in comparison to an individual who has not ingested the drug, but may have been unknowingly or knowingly (e.g. narcotics officer) exposed to a drug in their environment. It is also important to determine 1) if an individual with a given type of hair color or ethnicity type has a greater susceptibility to a positive hair drug test subsequent to environmental exposure; 2) if hygienic treatments affect the

positive hair drug test results; 3) if susceptibility of hair to drug environmental exposures can be qualitatively determined through hair characterization studies, such as morphological analysis using microscopy techniques; and 4) if laboratory decontamination procedures can minimize the effects of environmental exposure enough to properly identify a drug user from an environmental contaminated individual.

Several studies have indicated the risk of occupational health hazards to first responders to methamphetamine clandestine laboratories, including law enforcement (Irvine and Chin, 1991; Martyny, 2007; McFadden et al., 2006). The persistence of the drug and chemicals of clandestine laboratories became such a public concern that in 2006 the Drug Enforcement Administration created a national registry (http://www.justice.gov/dea/seizures/index.html) posting "locations in each state where known methamphetamine clandestine laboratories or dumpsites are located so that individuals can be aware of possible meth contaminated sites within their communities" (http://www.justice.gov/dea/pubs/states/newsrel/wdo120506.html) (U.S. DEA, 2010). Beyond the occupational health hazards, first responders may also have the occupational hazard of testing positive for a workplace drug test when their job places them in an environment where drug activities occurred.

The results of this study are consistent with RTI's previously published results for contamination of hair with pharmaceutical-grade COC (Stout et al., 2006). In the prior study, all three COC sources resulted in significant quantities of COC being present on the hair and remaining there over the course of 10 weeks. The COC analytes were resistant to removal by hygienic treatment or by laboratory decontamination; however, there was a significant decline in the content of COC over the course of the study. Similarly, BE/COC ratios increased significantly over the course of the study period. While the previous study did not investigate the

effect of hair color and type with an adequate statistical power, this study used the same *in vitro* surface contamination model to evaluate hair color and type on COC concentration in hair and laboratory decontamination washes. In addition, the previous study did not evaluate additional assessment criteria; mainly, the effectiveness of decontamination wash procedures or the use of mathematical wash criterion.

Our current results suggest that the hair characterization studies such as morphological analysis using microscopy techniques could not qualitatively establish a relationship between the susceptibility of hair to drug environmental exposures of drug and morphological features. SEM analysis, bright field light microscopy, and incidence fluorescence microscopy with methylene blue and rhodamine B staining showed that interindividual variation was large for all hair types and ethnic groups. There were no obvious morphological features by any microscopy method used that demonstrated an obvious relationship to ethnicity or to hair color. While fluorescence microscopy allowed for visualization of staining patterns in opaque hair strands, the staining patterns were not clearly related to the ultimate concentrations of target analytes observed or with the likelihood of the hair sample being called drug positive by any criteria we investigated.

In this study, methanolic decontamination was not very effective at removing COC analytes from the surface of the hair following this *in vitro* contamination model. In contrast, the extended phosphate decontamination resulted in significantly lower concentrations of all targets.

Within each decontamination method, after methanolic decontamination, AA hair had more COC compared to BC hair ($p=0.015$) and DC hair ($p=0.046$), but DC and BC hair were not significantly different. AA hair had significantly greater CE content than only BC hair ($p=0.0054$), and NCOC was also greater in AA hair than BC hair ($p=0.009$)

After phosphate decontamination, AA hair had greater concentrations of COC than BC hair (p=0.015), but not DC hair. For CE, again, AA hair had greater concentrations than only BC hair (p=0.019). AA individuals had significantly lower BE/COC ratios than BC (p=0.0007) and DC (p=0.015) after methanolic decontamination and also after phosphate decontamination, though the significance of the difference between AA and DC hair was marginal (p=0.05). In the current study, the ratio exceeded 0.05 by Day 28 for BC individuals only as a group, and AA hair as a group average never exceeded the 0.05 ratio, though some individuals did. Overall, the extended phosphate decontamination resulted in far fewer positive hair results at early time points, but it did not entirely eliminate positive results. This *in vitro* contamination model suggests no relationship of positivity and ethnicity exists in situation of environmental COC surface contamination and exposure.

For the nine decision criteria investigated in this study, it is important to note that RTI applied these criteria to hair specimens because a reference laboratory would apply Criteria 1–3 under the proposed federal Mandatory Guidelines, and additional CE/COC and NCOC/COC ratios were also under investigation as part of this study. In other words, the reference laboratory would have had to analyze the specimens and applied the cutoffs directly to these results to determine if a hair sample was positive for COC. As shown in Table 3-12, a substantial number of analyzed specimens would have been determined as positive by many of the BE and CE criteria applied; however, this is greatly reduced following an extended phosphate buffer decontamination and application of a wash criterion. As Schaffer and colleagues (2007) have noted in several publications (Cairns et al., 2004a; 2004b), they have applied various ratios of compounds and used various mathematical calculations using the amounts of a drug found in the last wash solution. As noted by Kidwell and Smith (2006), this wash criterion has evolved over

the years. The proposed Mandatory Guidelines (SAMHSA, 2004) do not have a provision for the use of such criteria, but our results indicate that use of such a wash criterion in hair drug testing may be a needed layer of separating a drug-user from a non-user.

The application of a decontamination wash and wash criterion may also assist with the elimination of a "color bias" or "ethic bias" because this simple and conservative "clinical or assessment adjustments" may be enough to modify "the threshold values and negate the hair color contribution," as proposed by Mieczkowski and colleagues (2007). Since the outcome of possible multiple biases is not known, further research is needed to determine whether the variables can be adequately controlled and resolved through analytical techniques employed for hair testing for drugs of abuse.

As noted by Schaffer and colleagues (2007) in a letter to the editor in *Journal of Analytical Toxicology* of RTI's previous research, this study design is an *in vitro* model, and it has limitations because of its use. However, the *in vitro* model allows for exposures and controls that would not be possible in an *in vivo* model. Schaffer and colleagues have also criticized that the quantity of COC used in this *in vivo* model is unrealistic because it is not clearly understood how much COC may be on a surface that is touched by law enforcement or others. Some research with methamphetamine cook houses suggests that high surface contamination may be possible. Although this quantity of COC we used may be too large for some scenarios, it may be too small to be representative of other scenarios.

3.5.1 Implications for Policy and Practice

The work performed under this study may directly affect the use of hair testing in a variety of investigatory applications and criminal justice systems. Many of its implications for policy and practice complement prior hair drug testing research at RTI.

The results of this study will influence how hair-testing results are interpreted and could significantly impact whether national agencies continue to use hair testing in their drug-free workplace programs and criminal justice applications. These results may directly affect policy implementation for drug-facilitated crimes (e.g., drug-facilitated sexual assault, homicide by drug poisoning, child abuse by drug poisoning) and parole and rehabilitation compliance. By contributing evidence through *in vitro* environmental COC contamination studies supporting the use of more stringent laboratory decontamination methods and wash criterion calculation can minimize and even eliminate any potential "color" or "ethnic bias" in hair drug testing.

Likely, the most significant impact of this research will be the need for hair drug testing guidelines to require the use of extended decontamination wash procedures and mathematical calculations, in addition to currently used cut-off concentrations and the BE/COC ratio. The current proposed guidelines do not include either a requirement for decontamination or the ability to use any other assessment criteria. These results suggest that current guidelines are not sufficient to discriminate potential environmental contamination from usage of COC or the natural accumulation of COC analytes in darker hair. Although it is unlikely that widespread contamination of hair is an issue, the federal workplace drug-testing program includes individuals who may have exposure to high drug concentrations because of their jobs. Therefore, the proposed Mandatory Guidelines for hair testing may need to be amended by adding an additional specimen preparatory step for COC analysis that would include a requirement for a

laboratory to decontaminate hair specimens prior to analysis. Furthermore, potential criteria to evaluate the decontamination solutions in relation to the hair concentrations may be necessary unless the presence of other unique COC metabolites in hair can be reliably established. These laboratory adjustments are very much like administrative concentration cutoffs used in clinical and drug testing laboratories routinely. Until such a time that these multiple biases of an individual's hair are more fully understood, these analytical steps may protect individuals from the issues of environmental contamination and intrinsically greater drug deposition into darker hair.

3.5.2 Implications for Further Research

These results suggest the need for a decontamination strategy to be used by laboratories that conduct hair testing to detect COC analytes. Our studies looked at two decontamination strategies and demonstrated that an extended phosphate buffer wash was more effective at removing COC analytes from the surface of environmentally contaminated hair. This study did not investigate other decontamination procedures currently reported in the literature (e.g., organic solvent washes), and continued research may determine other effective decontamination strategies. Further work to examine the efficacy of decontamination procedures is essential, and it would include not only the use of decontamination strategies, but also the evaluation of the hair-drug concentrations in comparison to the drug detected in the decontamination solvents. The criteria for both known contaminated and drug-user hair specimens subjected to these decontamination schemes also needs to be evaluated.

The extent of surface contamination is poorly understood for environments where exposures may occur, such as for law enforcement who work in areas where there is known drug usage. Better estimation of the extent of surface contamination and the quantities of drug that

could be realistically transferred to hair are central components to understanding if contamination is a problem. Similar to Martyny and colleagues' studies (2008a) with methamphetamine determinations in clandestine laboratories and other contaminated areas, it is important to measure the likelihood of environmental exposure to a drug for employees who are in a workplace that exposes them (e.g., narcotics officer, first responders, pharmaceutical laboratory personnel). Moreover, RTI's *in vitro* model is a surface contamination using COC powder and does not investigate aerosolization of the drug, which is also a realistic means of environmental contamination. This information is key to resolving if *in vitro* contamination models are using quantities of COC that are too low or too high to be realistic and if environmental contamination is an issue for individuals who are not drug users, but who are exposed to a drug.

Other drugs, including heroin and methamphetamines, as well as other opioids, may also cause contamination problems for hair because drug use sometimes occurs by crushing the tablets and snorting the powder. Limited research is available on the potential impact of transferred contamination and its impact on analysis of specific drugs. These COC results do not necessarily imply that all drugs will interact with hair so strongly and be so difficult to discriminate, but they do indicate that there is significant possibility of a similar potential problem for other drugs.

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6. DISSEMINATION OF RESEARCH FINDINGS

The research findings of this project have been presented at the following annual professional meetings:

1. Ropero-Miller JD, Stout PR, Shipkowski, K, Mitchell JM, Baylor MR, Doorn SS, Crankshaw OS. Ultrastructural Evaluation of Human Hair by Microscopy for the Determination of Morphological Differences. Society of Forensic Toxicology. October 18–22, 2010, Richmond, VA.

2. Ropero-Miller JD, Lewallen C, Bynum NB, Meaders M, Espenshade J, Mitchell JM, Baylor MR, Stout PR. Analysis of Cocaine Analytes in Human Hair II: Evaluation of Different Hair Color and Ethnicity Types Following Surface Contamination and

Laboratory Decontamination. American Academy of Forensic Sciences. February 21–26, 2011. Chicago, IL.

3. Ropero-Miller JD, Stout PR, Shipkowski, K, Mitchell JM, Baylor MR, Doorn SS, Crankshaw OS. Analysis of Cocaine Analytes in Human Hair: Ultrastructural Evaluation of Human Hair by Microscopy for the Determination of Morphological Differences Following Surface Contamination and Laboratory Decontamination. American Academy of Forensic Sciences. February 21–26, 2011. Chicago, IL.

In addition, RTI has made the dissemination of these research findings a priority goal of this project. The following publications will result from this final report and will be submitted to a professional journal:

4. Stout, P.R., J.D. Ropero-Miller,. 2011 Morphological changes in human head hair subjected to various drug testing decontamination strategies II: Can These Changes Predict A Correlation between Hair Color and Cocaine Concentration? *Forensic Science International*.
5. Ropero-Miller, J.D., P.R. Stout. 2011. Cocaine Analytes in Human Hair II: Analysis of Cocaine Analytes in Human Hair II: Evaluation of Different Hair Color and Ethnicity Types. *Forensic Science International.*

Appendix A

Examples of RTI Institutional Review Board (IRB) Forms

Consent to Participate in Research

Youth Assent for Children Aged 7–10 to Participate in a Research Study

Youth Assent for Children Aged 11–17 to Participate in a Research Study

Participant's initials: _____ Date:_____ Consenter's initials:_____ Date:_____

Consent to Participate in Research

Title of Research: Evaluate cocaine analytes in hair of different color (e.g., light, dark) and ethnic origin.

Introduction

We are asking you to be in a research study. Being in this study is voluntary. To make an informed judgment on whether or not you want to be part of this study, you should understand the risks and benefits of participating. This process is known as informed consent.

This consent form gives you detailed information about the research study. Please ask any questions you may have about the study or this form before signing it. We will give you a copy of the consent form to keep.

Purpose

The Analysis of Cocaine Analytes in Human Hair II: Evaluation of Different Hair Color and Ethnicity Types Following Surface-Contamination and Laboratory Decontamination is a research study paid for by a grant from the National Institute of Justice. The study is being conducted by RTI International, a research organization located in Research Triangle Park, North Carolina. The purpose of this study is to evaluate the incorporation into and detection of drugs of abuse in hair of different color (e.g., light, dark) and ethnic origin (e.g., Caucasian, African American). You are one of approximately 36 people being asked to participate in this study.

Procedures

Before collection you will be asked to complete an informal interview in a private setting to determine if you qualify for the study. If you agree to participate, you will be asked to answer several basic questions about if you use or are exposed to cocaine and how you treat your hair and to provide a large sample of head hair. We will also ask about drug usage history as it pertains to potential exposure to cocaine (whether that the person is a user or lives with a known user) as this would make the sample ineligible. This information will not be documented. If you have short hair (roughly 3 inches) an adequate sample will involve shaving you head with electric clippers. If you have longer hair, we may be able to get an adequate sample clipping a portion of hair close to the scalp from the back of your head or a "ponytail" clip with scissors. Only demographic information including age, gender and hair treatment history will be collected and documented with the samples. Once the study is completed the samples will be destroyed.

Biospecimen Collection

You will be asked to provide a large sample of hair (about 10 grams, or about 0.5 oz). Collection of these samples will involve clipping the hair. For someone with short hair (less than 3 inches in length) this could

Participant's initials: _____ Date:_____ Consenter's initials:_____ Date:_____

involve shaving the head with electric clippers or for someone with longer hair (6+ inches) this could involve taking a bundle of approximately 0.5 inch diameter from underneath the hair or a "ponytail" clip with scissors. This is non-invasive and is the same as getting a hair cut.

Your samples will be used only for research and will not be sold. New research laboratory tests performed on your sample may result in inventions, products, or procedures that have commercial value and are eligible for protection by a patent. You will not be eligible for any further compensation resulting from patented research.

Study Duration

Your participation in the brief interview will take about 5 minutes. Collection of the hair will take about 15 minutes.

Possible Risks or Discomforts

Risks from the hair collection: The hair collection is the same as getting a hair cut, your hair will be preferably be cut close to the scalp but NOT pulled out.

No personally identifiable information will be kept beyond your consent on this form. Your hair will be assigned a number and only associated with the demographic information collected.

Benefits

Your Benefits There are no direct benefits to you from participating in this study.

Benefits for Other People The results of this study will help forensic scientists better understand drug testing results in hair making these test more reliable.

Payment for Participation

You will receive $500 for providing a hair sample for the study. A cash payment will be issued after the hair is cut and weighed.

Confidentiality

We have taken many precautions to protect your information. Your name will be replaced with a number. Other personal information such as ethnicity and contact information will be stored separately from the answers you provide on the consent form. If the results of this study are presented at scientific meetings or published in scientific journals, no information will be included that could identify you or your answers personally.

Information from this study may be given to persons or companies which are contracted by RTI or the sponsor to have access to the research information during and after the study. It is possible that RTI may need to release your name to another party, but this is very unlikely. If this happens, we would release your name but would not release any of your other information.

The Institutional Review Board (IRB) at RTI International has reviewed this research. An IRB is a group of people who are responsible for assuring that the rights of participants in research are protected. The IRB

Participant's initials: _____ Date:_____ Consenter's initials:_____ Date:_____

may review the records of your participation in this research to assure that proper procedures were followed. A representative of the IRB may contact you for information about your experience with this research. This representative will be given your name, but will not be given any of your confidential study data. If you wish, you may refuse to answer any questions this person may ask.

Future Contacts

You will not contact by the Principal Investigators or any of their staff in the future.

Your Rights

Your decision to take part in this research study is completely voluntary. You can refuse any part of the study and you can stop participating at any time. You can refuse to answer any question. If you decide to participate and later change your mind, you will not be contacted again or asked for further information

Your Questions

If you have any questions about the study, you may call Dr. Jeri Ropero-Miller at (866) 252-8415. If you have any questions about your rights as a study participant, you may call RTI's Office of Research Protection at 1-866-214-2043 (a toll-free number).

YOU WILL BE GIVEN A COPY OF THIS CONSENT FORM TO KEEP.

Your signature below indicates that you have read the information provided above, have received answers to your questions, and have freely decided to participate in this research. By agreeing to participate in this research, you are not giving up any of your legal rights.

_____ _____

Date Signature of Participant

_____

Printed Name of Participant

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above-named individual.

_____ _____

Date Signature of Person Obtaining Consent

_____

Printed Name of Person Obtaining Consent

RTI International
Youth Assent for Children Aged 7-10 to Participate in a Research Study

---

Name of Youth:_____

Name of Parent/Legal Guardian:_____

My name is _____ and I work for a company called RTI International. We are doing a study on cocaine in hair and need hair without any drugs in it for our study. We are asking people to give a big chunk of hair (if their hair is long enough) or to have their head shaved to give enough hair for our study. We will not pull your hair out and it will be like having a hair cut.

_____ said it was okay for you to talk with me about this study and to give your hair. If it is okay with you, I would like to ask you some questions and take a hair sample.

The answers you give will be kept totally private. No one outside of the research team and your parents will know your answers.

If you don't want to talk to me, that is okay. If you don't want to give a hair sample, that is also okay. If you would like to take a break at any time, just tell me.

Our talk today will last no longer than 5 minutes. You will be given a copy of this form to keep. When we finish, I will give you $500 for taking the time to talk with me and giving a hair sample.

May we collect a hair sample:

____By shaving your head with electric clippers?

____By using scissors to cut a chunk off close to your skin from the back of your head or cut 3-5 inches from the ends ("ponytail" cut)? This is only possible if the project staff thinks your hair is long enough

_____Child agrees
_____Child does not agree
_____Child did not appear to understand explanation

_____

_____	_____	_____
Signature/Mark of Child	Signature of Interviewer	Date

RTI Project Number: 0211987
RTI IRB Approval Date: June 23, 2009
Assent Form Version Date: July 9, 2009

RTI International
Youth Assent for Children Aged 11-17 to Participate in a Research Study

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Name of Youth:_____

Name of Parent/Legal Guardian:_____

My name is _____ and I work for a company called RTI International. We are doing a study on cocaine in hair and need hair without any drugs in it for our study. We are asking people to give a big chunk of hair (if their hair is long enough) or to have their head shaved to give enough hair for our study. We will not pull your hair out and it will be like having a hair cut.

_____ has given permission for you to talk with me about this study and to give your hair. If it is okay with you, I would like to ask you some questions and take a hair sample.

Your participation in this study is completely voluntary. You can refuse to answer any question or to participate. You also have the right to stop the interview at any time

Anything you tell me is confidential. No one outside of the research team and your parents will know your answers.

Our talk today will last no longer than 5 minutes and the collection will only take about 15 minutes. You will be given a copy of this consent form to keep. When we finish, I will give you $500 for taking the time to talk with me and providing a hair sample.

May we collect a hair sample from you?

____By shaving your head with electric clippers?

____By using scissors to cut a chunk off close to your skin from the back of your head or cut 3-5 inches from the ends ("ponytail" cut)? This is only possible if the project staff thinks your hair is long enough.

_____Child agrees
_____Child does not agree
_____Child did not appear to understand explanation

_____

_____	_____	_____
Signature/Mark of Child	Signature of Interviewer	Date

RTI Project Number: 0211987
RTI IRB Approval Date: June 23, 2009
Assent Form Version Date: July 9, 2009

Appendix B

RTI Batch Contamination Record Template

PAGE 1 OF 1

RESEARCH TRIANGLE INSTITUTE
BATCH CONTAMINATION RECORD
National Institute of Justice (NIJ)
Cocaine Contamination Study (RTI Project No. 0211897; NIJ Grant Number 2008-DN-BX-K179)

TITLE: Application of cocaine HCL to hair sample for surface contamination study Batch 3 BCR
BCR format reviewed prior to production (date and sign): _____
Document Version (e.g.,: 2.12.17.09 for Ver. 2 on Dec. 17, 2009) _____7/19/10____

Remove the pre-weighed cocaine aliquots from the vault and bring to the laboratory where contamination is to take place. Each aliquot of cocaine (8 mg) will be applied to its corresponding hair specimen (10 g). Gloved hands will be sprayed with synthetic sweat solution and allowed to dry. The cocaine will be distributed on the palms of the gloved hands by gently rubbing until the cocaine hydrochloride (HCl) powder is no longer visible. Once the cocaine has been distributed on the hands, handle the hair for 5 minutes with the intent of evenly transferring the cocaine to the hair. The contaminated hair will be placed between two sheets of filter paper and allowed to rest for one hour. Repeat this process for the remaining 5 hair specimens. The contamination process will be done in batches of 6 hair samples for a total of 6 batches. The time of contamination, and date will recorded in the space provided below.

Aliquot ID For Contamination	Cocaine HCl Aliquot Number	Start Time	End Time	Date	Initials
BATCH I					
11367-152-10	25				
11367-152-15	26				
11367-152-16	27				
11367-152-17	28				
11367-152-20	29				
11367-152-29	30				
11367-152-31	31				
11367-152-33	32				
11367-152-37	33				
11367-152-41	34				
11367-152-45	35				
11367-152-49	36				
11367-152-50	37				

Note: Samples 11367-152-5, 11367-152-6, 11367-152-8, 11367-152-11, 11367-152-12, 11367-152-32, 11367-152-35, 11367-152-42, and 11367-152-44 have been removed form the study due to reference laboratory analysis. These samples will be replaced by samples 11367-152-37 and 11367-152-41 11367-152-43,11367-152-45, 11367-152-46 and 1367-152-47.

Record of Batch Analysts (sign & date):

Batch scientist I:_____and _____
Brief description of Nonconformance (initial & date):

_____
_____
_____

END OF PAGE 1 OF 1

PAGE 2 OF 2
Cocaine Surface Contamination
BCR
End of Batch Contamination Record

COMPLETED BCR REVIEWED BY Principal Investigator (PI/co-PI) (sign and date):

_____

END OF PAGE 2 OF 2

Appendix C

Composite Images from Each Microscopic Technique

Part 1: Individuals at Pre-contamination

Part 2: Individuals at Time Point 4 After Methanol Decontamination

Part 3: Individuals at Time Point 4 After Phosphate Buffer Decontamination

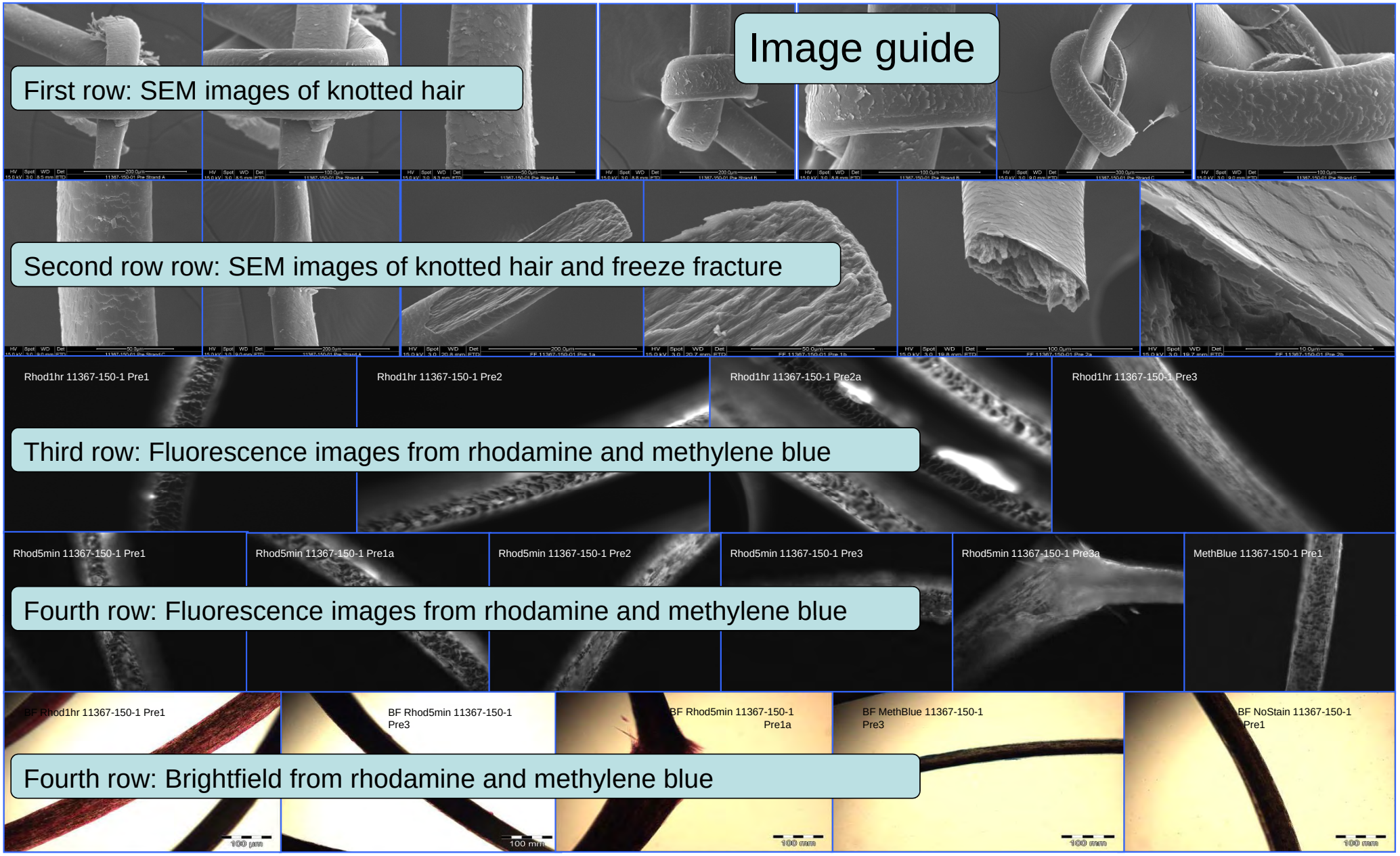
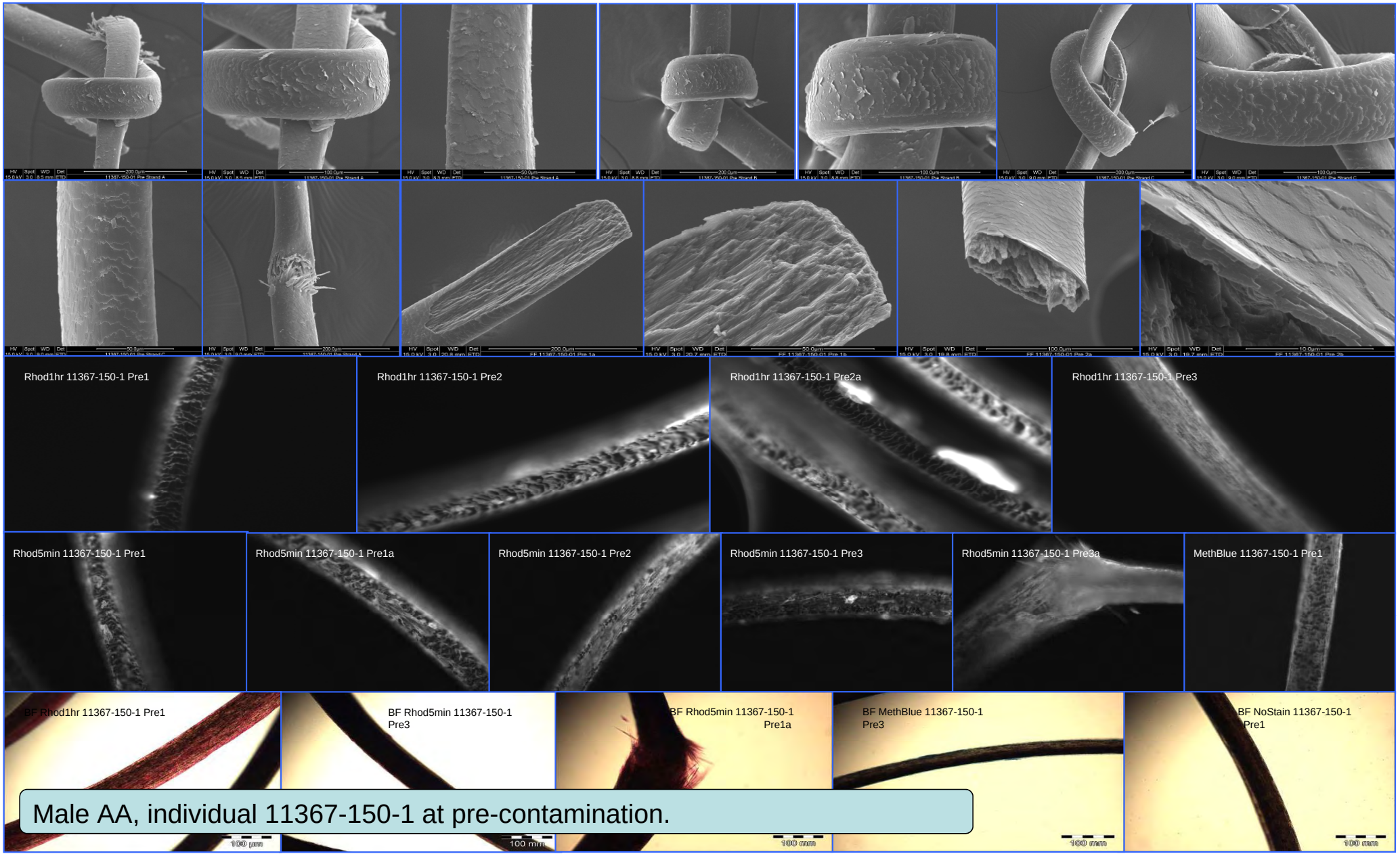
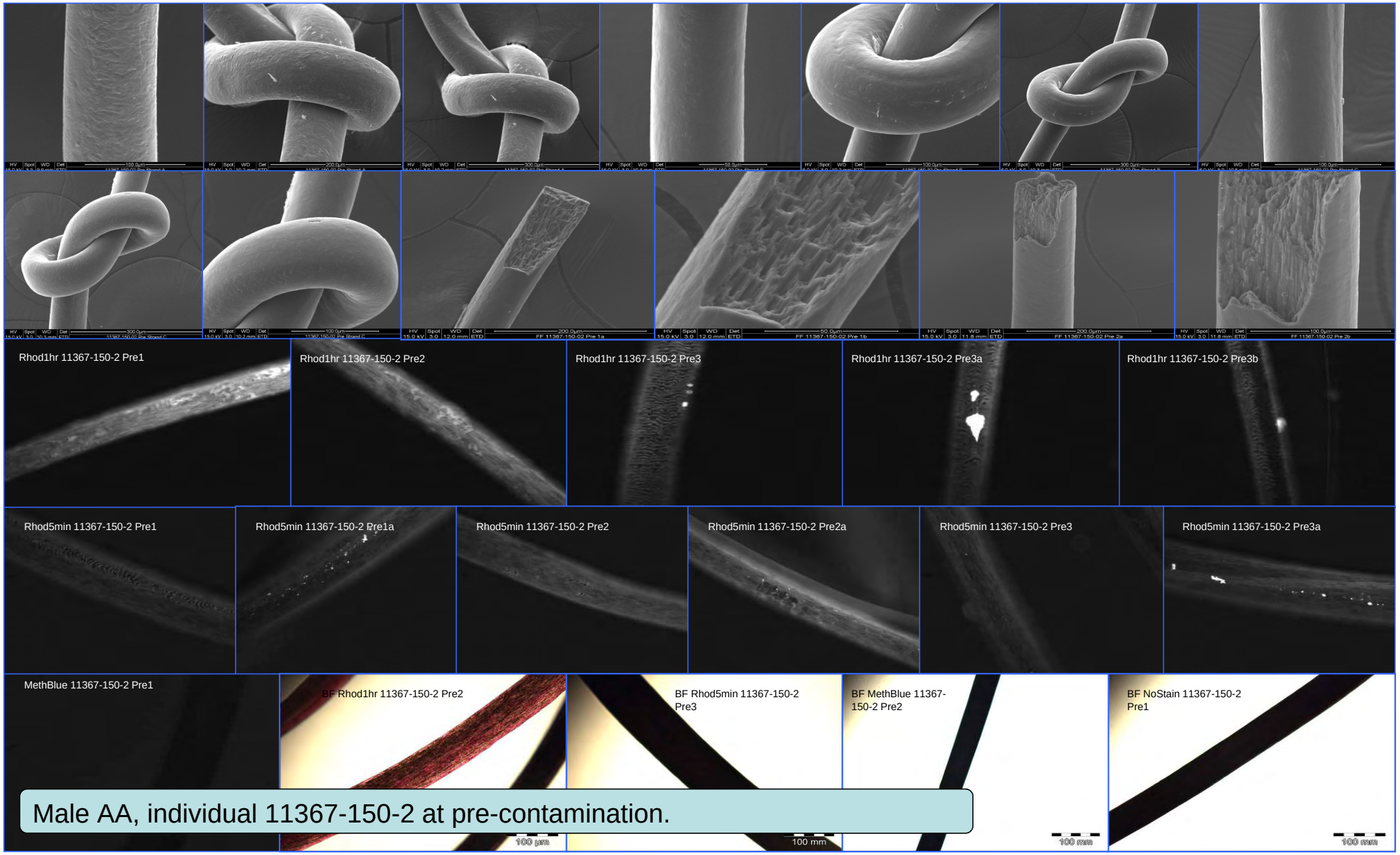


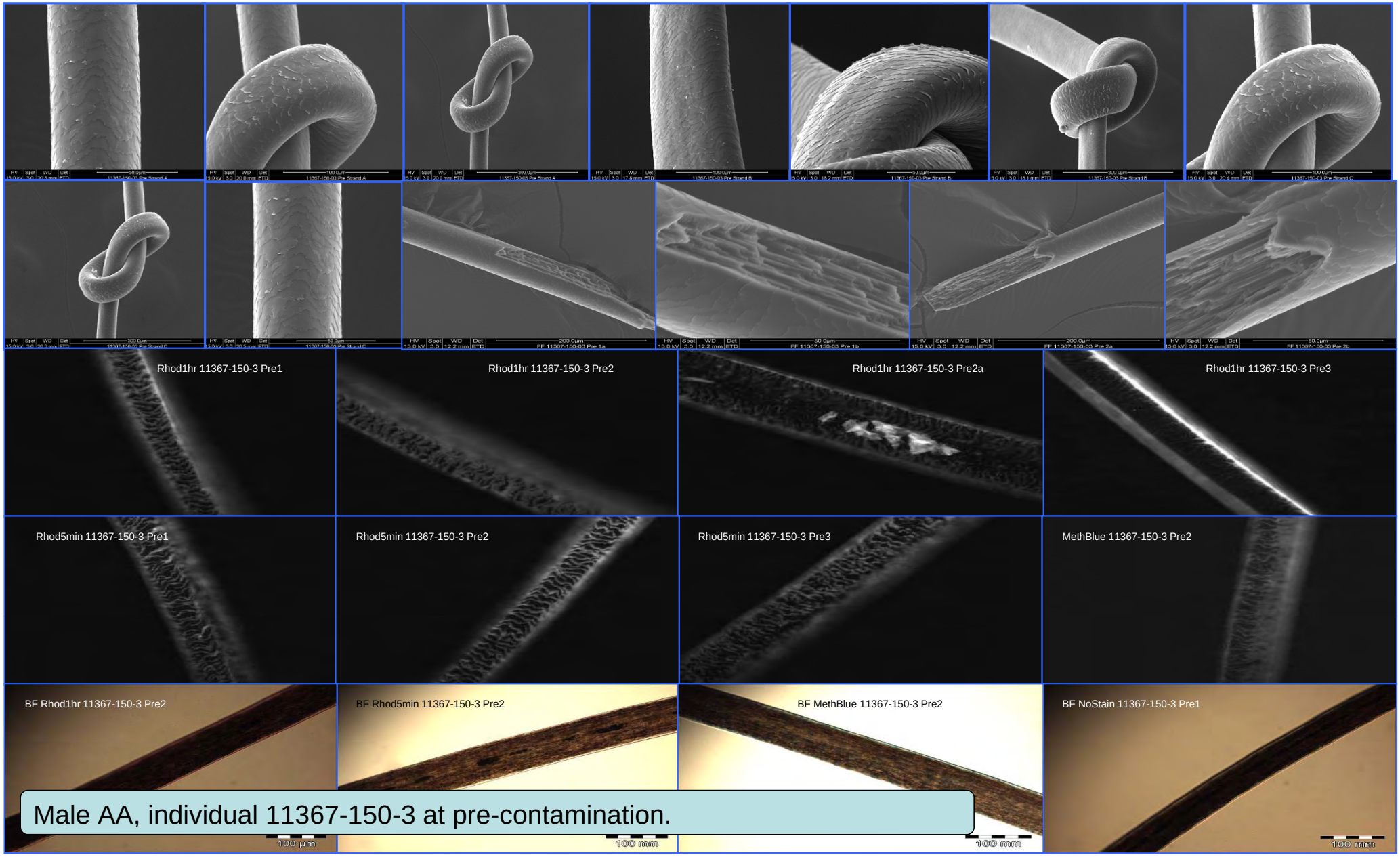
Image guide
First row: SEM images of knotted hair
Second row row: SEM images of knotted hair and freeze fracture
Rhod1hr 11367-150-1 Pre1
Rhod1hr 11367-150-1 Pre2
Rhod1hr 11367-150-1 Pre2a
Rhod1hr 11367-150-1 Pre3
Third row: Fluorescence images from rhodamine and methylene blue
Rhod5min 11367-150-1 Pre1
Rhod5min 11367-150-1 Pre1a
Rhod5min 11367-150-1 Pre2
Rhod5min 11367-150-1 Pre3
Rhod5min 11367-150-1 Pre3a
MethBlue 11367-150-1 Pre1
Fourth row: Fluorescence images from rhodamine and methylene blue
BF Rhod1hr 11367-150-1 Pre1
BF Rhod5min 11367-150-1 Pre3
BF Rhod5min 11367-150-1 Pre1a
BF MethBlue 11367-150-1 Pre3
BF NoStain 11367-150-1 Pre1
Fourth row: Brightfield from rhodamine and methylene blue

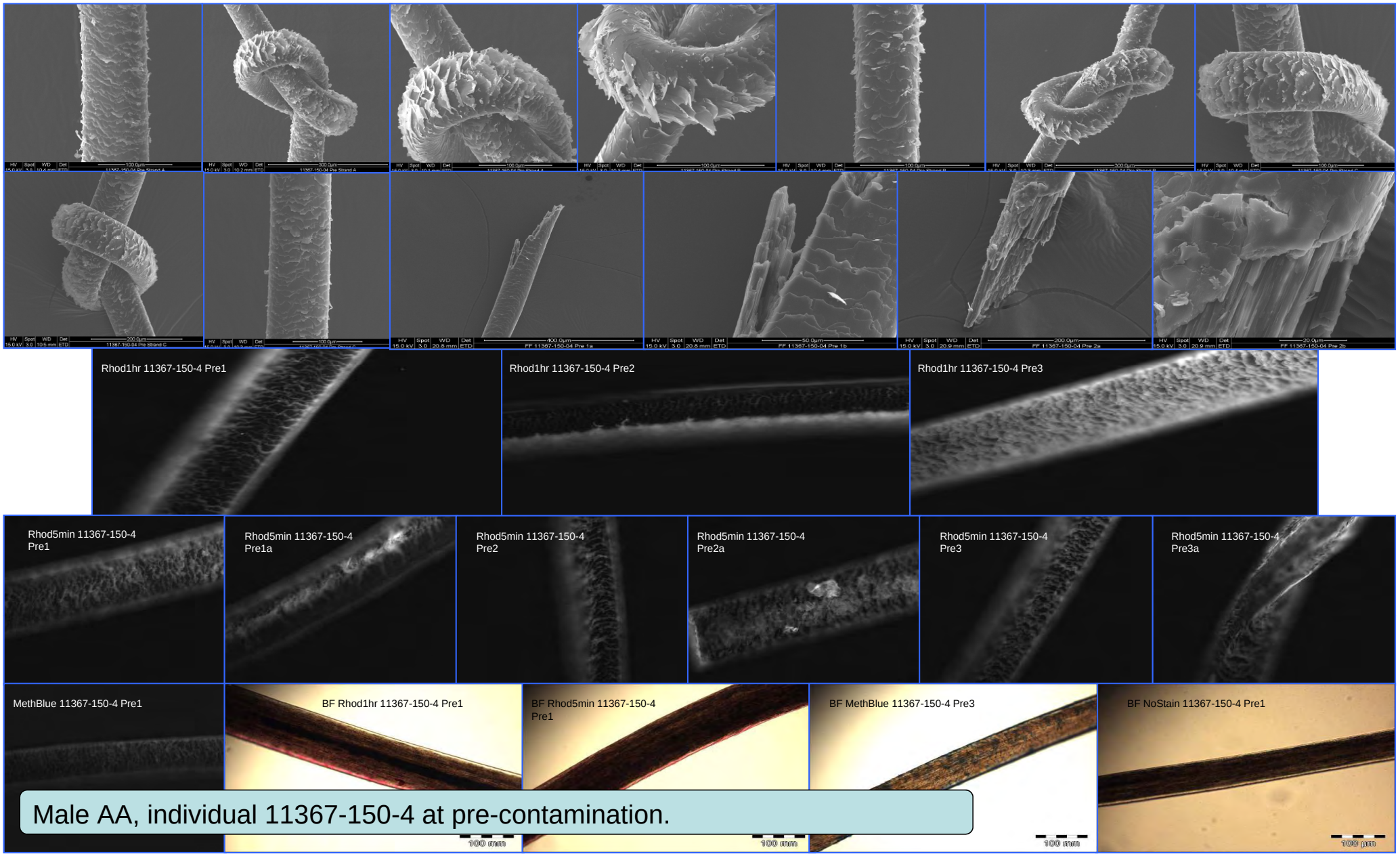
Appendix C, Part 1

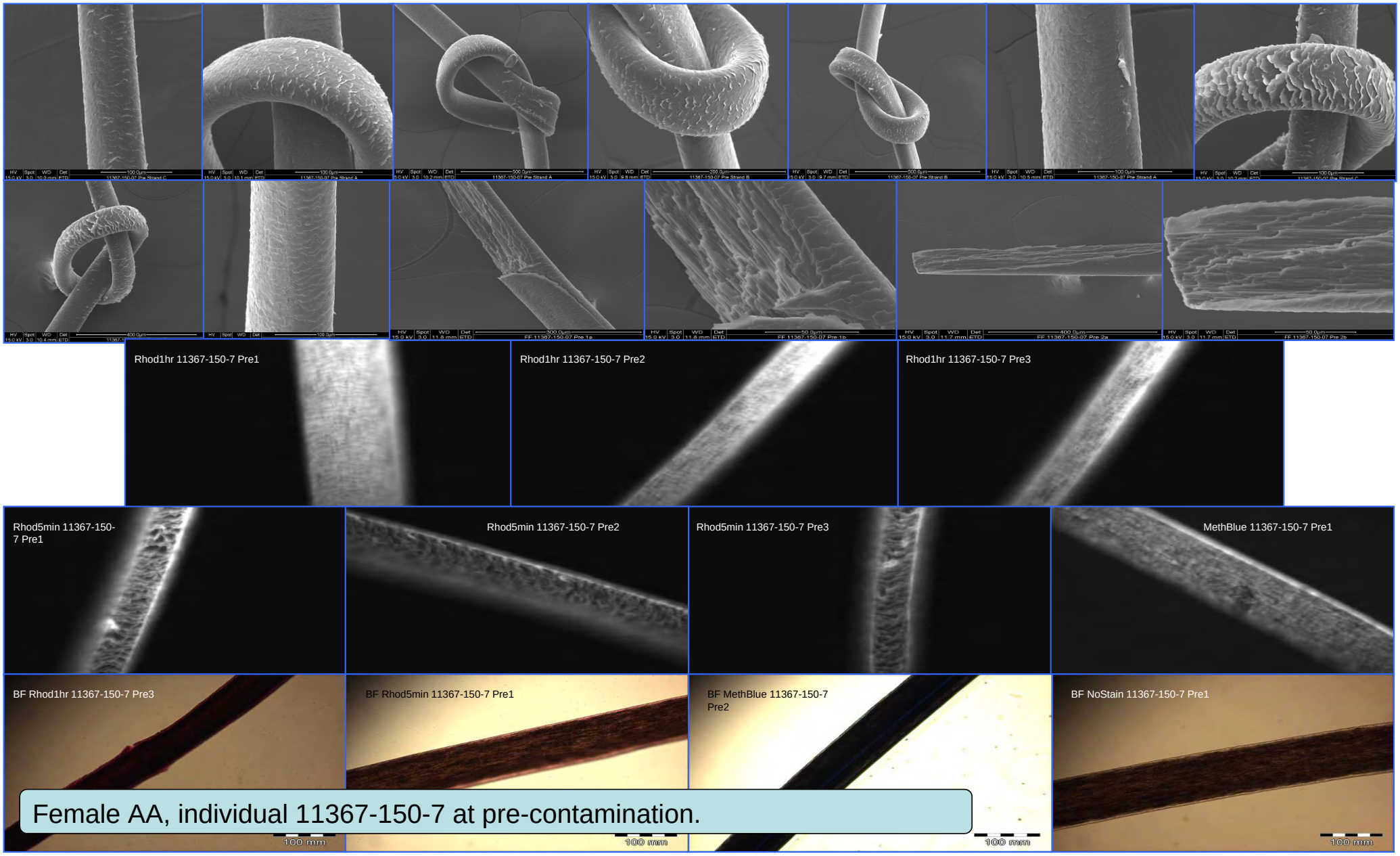
Individuals at Pre-contamination

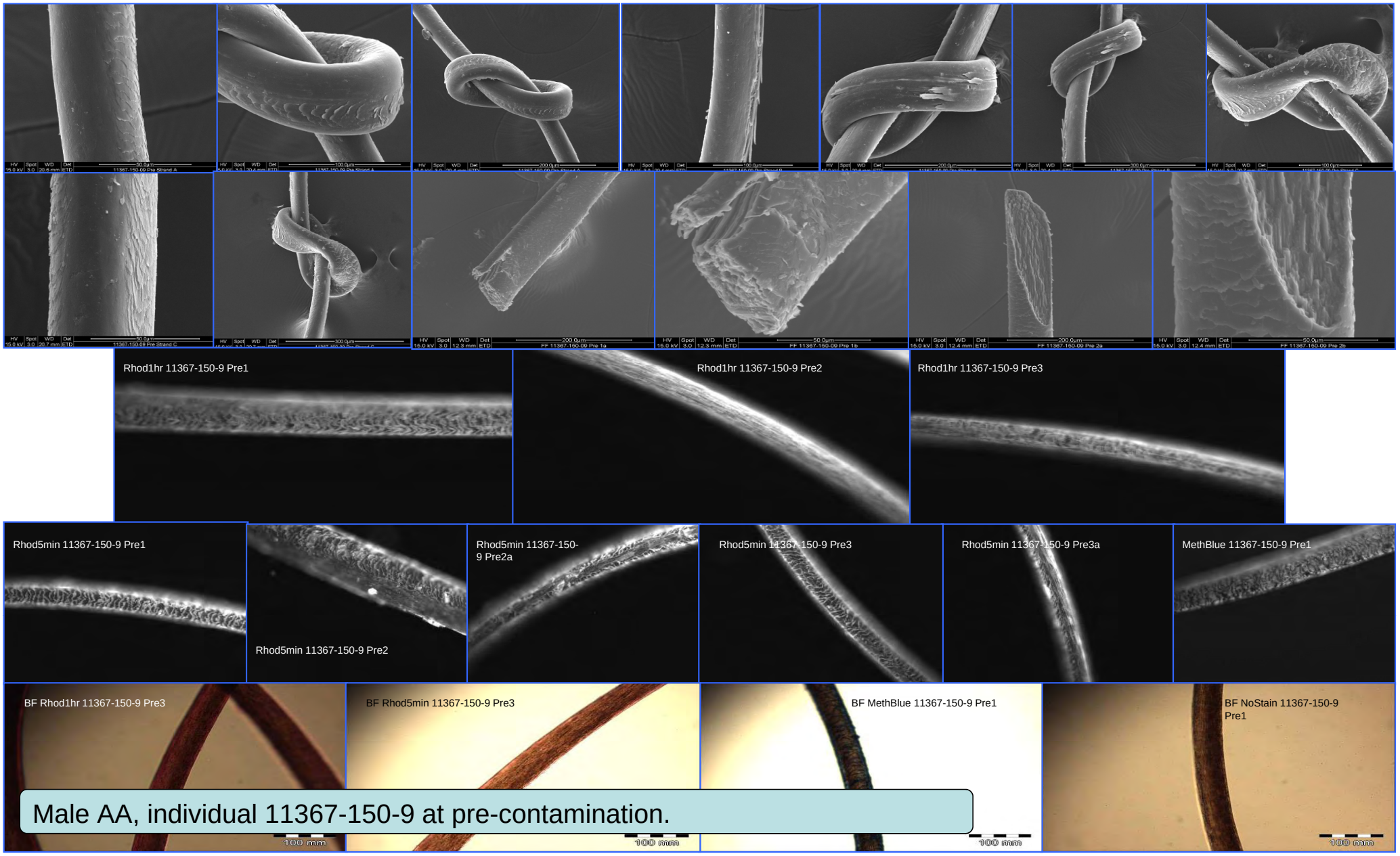

Rhod1hr 11367-150-1 Pre1
Rhod1hr 11367-150-1 Pre2
Rhod1hr 11367-150-1 Pre2a
Rhod1hr 11367-150-1 Pre3
Rhod5min 11367-150-1 Pre1
Rhod5min 11367-150-1 Pre1a
Rhod5min 11367-150-1 Pre2
Rhod5min 11367-150-1 Pre3
Rhod5min 11367-150-1 Pre3a
MethBlue 11367-150-1 Pre1
BF Rhod1hr 11367-150-1 Pre1
BF Rhod5min 11367-150-1 Pre3
BF Rhod5min 11367-150-1 Pre1a
BF MethBlue 11367-150-1 Pre3
BF NoStain 11367-150-1 Pre1
Male AA, individual 11367-150-1 at pre-contamination.

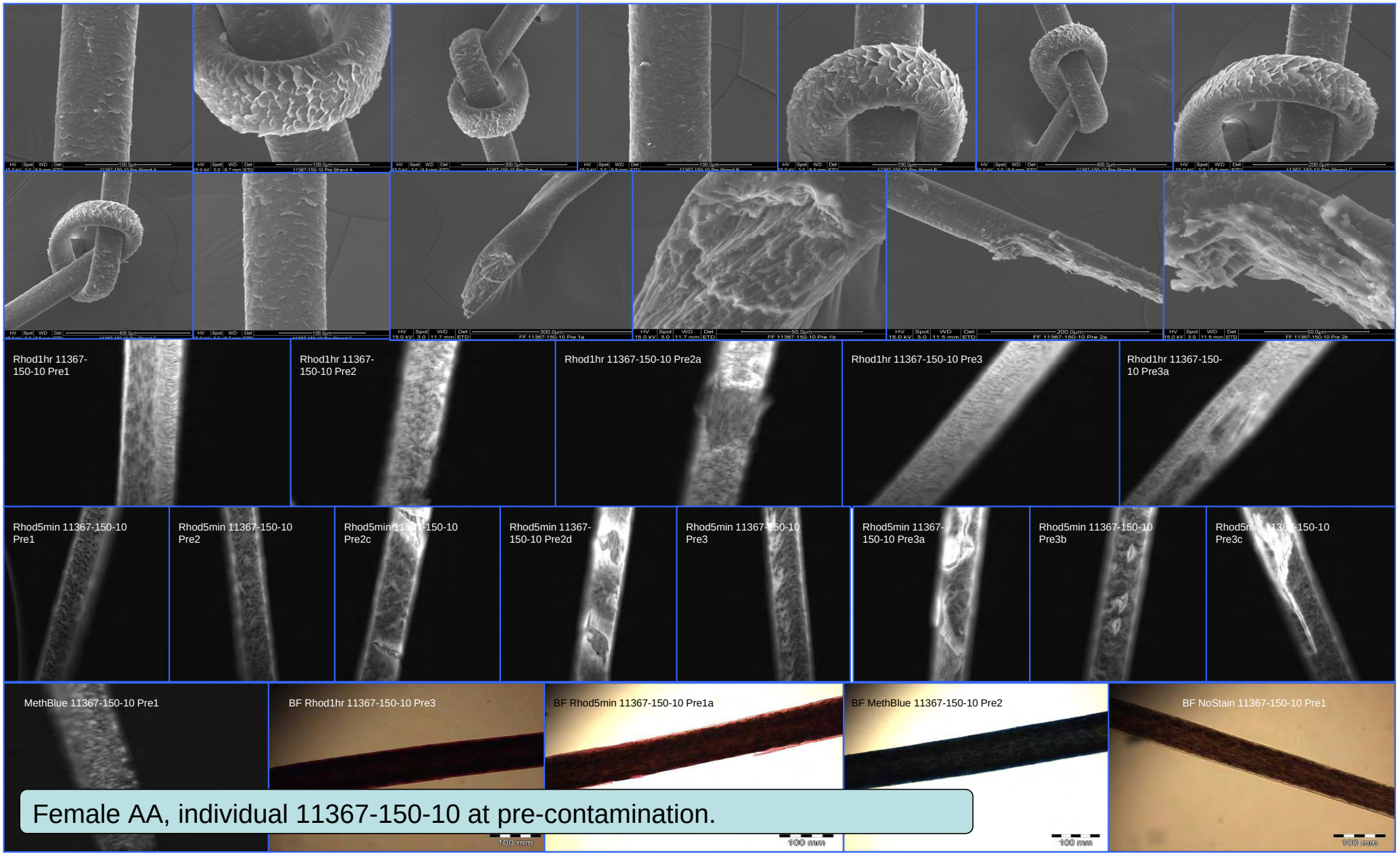

Rhod1hr 11367-150-2 Pre1
Rhod1hr 11367-150-2 Pre2
Rhod1hr 11367-150-2 Pre3
Rhod1hr 11367-150-2 Pre3a
Rhod1hr 11367-150-2 Pre3b
Rhod5min 11367-150-2 Pre1
Rhod5min 11367-150-2 Pre1a
Rhod5min 11367-150-2 Pre2
Rhod5min 11367-150-2 Pre2a
Rhod5min 11367-150-2 Pre3
Rhod5min 11367-150-2 Pre3a
MethBlue 11367-150-2 Pre1
BF Rhod1hr 11367-150-2 Pre2
BF Rhod5min 11367-150-2 Pre3
BF MethBlue 11367-150-2 Pre2
BF NoStain 11367-150-2 Pre1
100 µm
100 mm
100 mm
100 mm
Male AA, individual 11367-150-2 at pre-contamination.

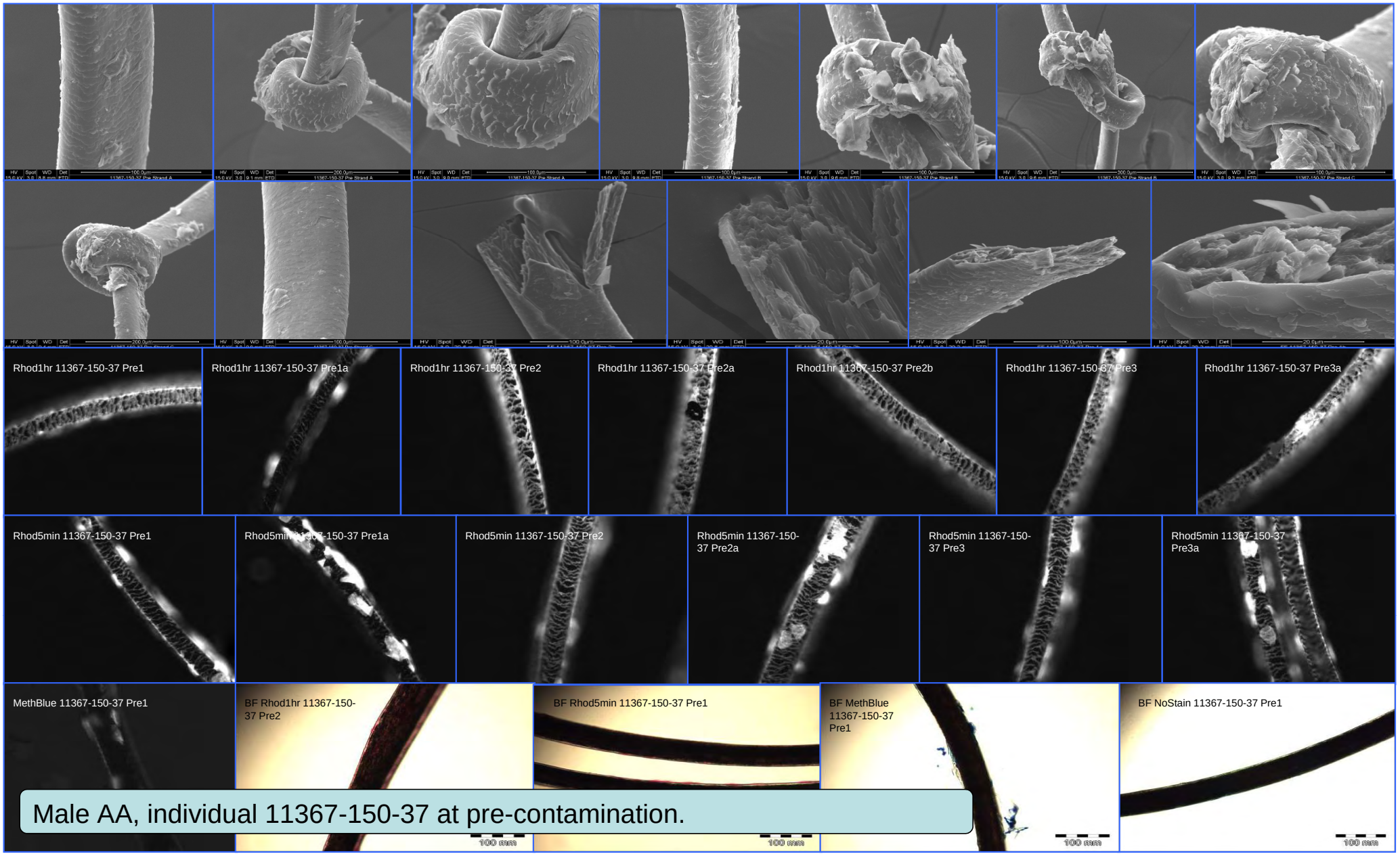

Rhod1hr 11367-150-3 Pre1
Rhod1hr 11367-150-3 Pre2
Rhod1hr 11367-150-3 Pre2a
Rhod1hr 11367-150-3 Pre3
Rhod5min 11367-150-3 Pre1
Rhod5min 11367-150-3 Pre2
Rhod5min 11367-150-3 Pre3
MethBlue 11367-150-3 Pre2
BF Rhod1hr 11367-150-3 Pre2
BF Rhod5min 11367-150-3 Pre2
BF MethBlue 11367-150-3 Pre2
BF NoStain 11367-150-3 Pre1
100 µm
100 mm
100 mm
100 mm
Male AA, individual 11367-150-3 at pre-contamination.

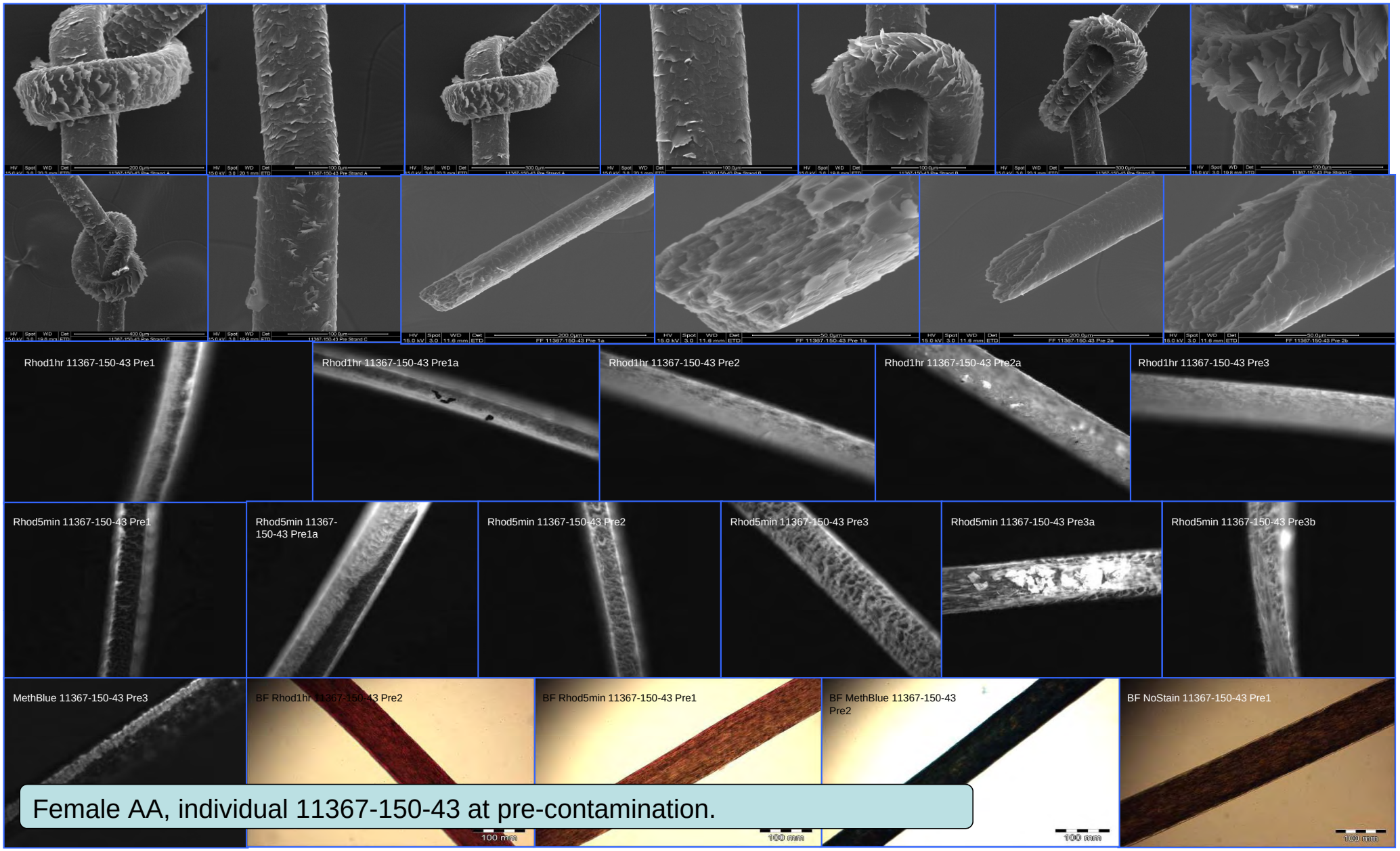

Rhod1hr 11367-150-4 Pre1
Rhod1hr 11367-150-4 Pre2
Rhod1hr 11367-150-4 Pre3
Rhod5min 11367-150-4 Pre1
Rhod5min 11367-150-4 Pre1a
Rhod5min 11367-150-4 Pre2
Rhod5min 11367-150-4 Pre2a
Rhod5min 11367-150-4 Pre3
Rhod5min 11367-150-4 Pre3a
MethBlue 11367-150-4 Pre1
BF Rhod1hr 11367-150-4 Pre1
BF Rhod5min 11367-150-4 Pre1
BF MethBlue 11367-150-4 Pre3
BF NoStain 11367-150-4 Pre1
100 mm
100 mm
100 mm
100 µm
Male AA, individual 11367-150-4 at pre-contamination.


Rhod1hr 11367-150-7 Pre1
Rhod1hr 11367-150-7 Pre2
Rhod1hr 11367-150-7 Pre3
Rhod5min 11367-150-7 Pre1
Rhod5min 11367-150-7 Pre2
Rhod5min 11367-150-7 Pre3
MethBlue 11367-150-7 Pre1
BF Rhod1hr 11367-150-7 Pre3
BF Rhod5min 11367-150-7 Pre1
BF MethBlue 11367-150-7 Pre2
BF NoStain 11367-150-7 Pre1
100 mm
Female AA, individual 11367-150-7 at pre-contamination.

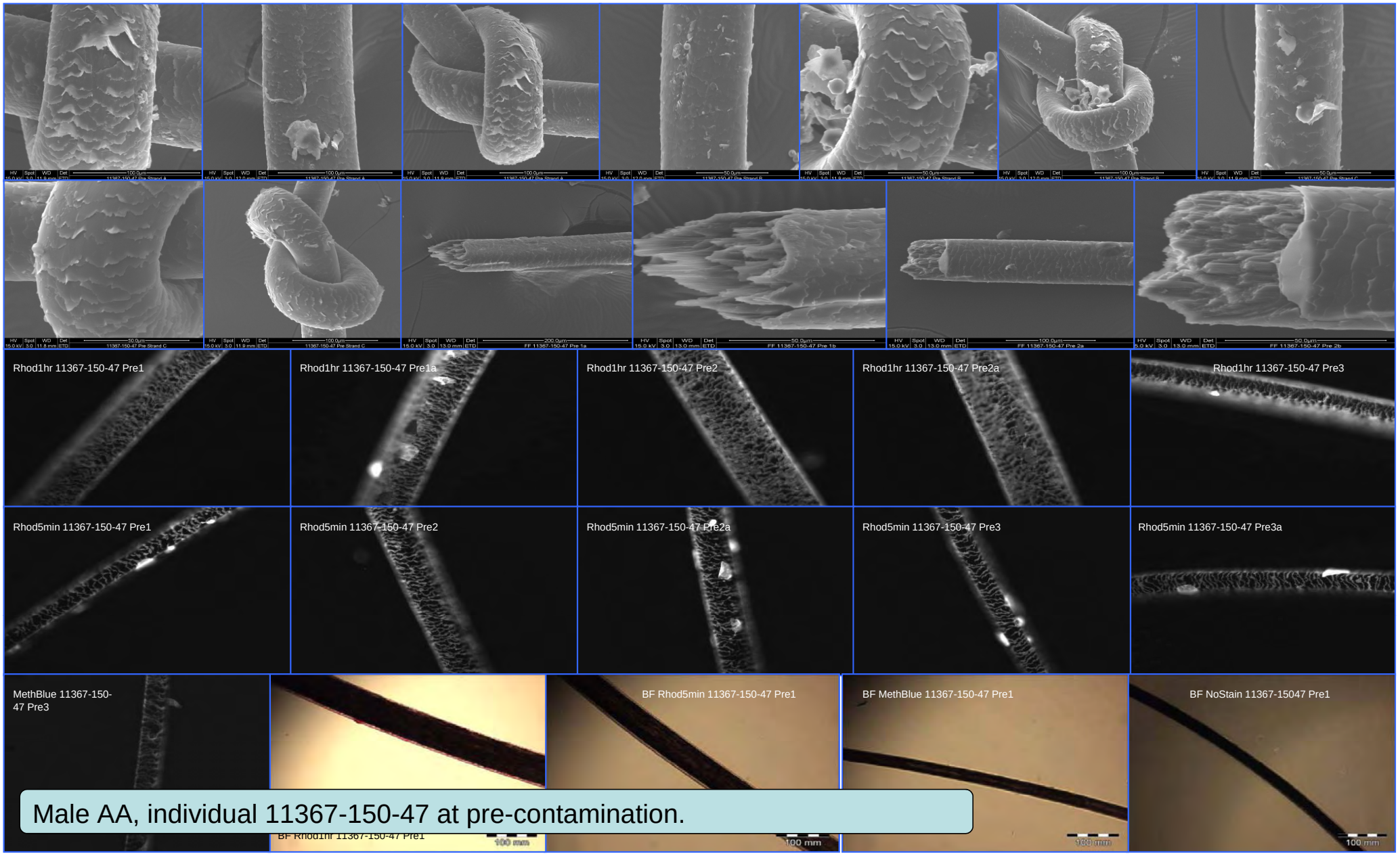

Rhod1hr 11367-150-9 Pre1
Rhod1hr 11367-150-9 Pre2
Rhod1hr 11367-150-9 Pre3
Rhod5min 11367-150-9 Pre1
Rhod5min 11367-150-9 Pre2
Rhod5min 11367-150-9 Pre2a
Rhod5min 11367-150-9 Pre3
Rhod5min 11367-150-9 Pre3a
MethBlue 11367-150-9 Pre1
BF Rhod1hr 11367-150-9 Pre3
BF Rhod5min 11367-150-9 Pre3
BF MethBlue 11367-150-9 Pre1
BF NoStain 11367-150-9 Pre1
100 mm
Male AA, individual 11367-150-9 at pre-contamination.

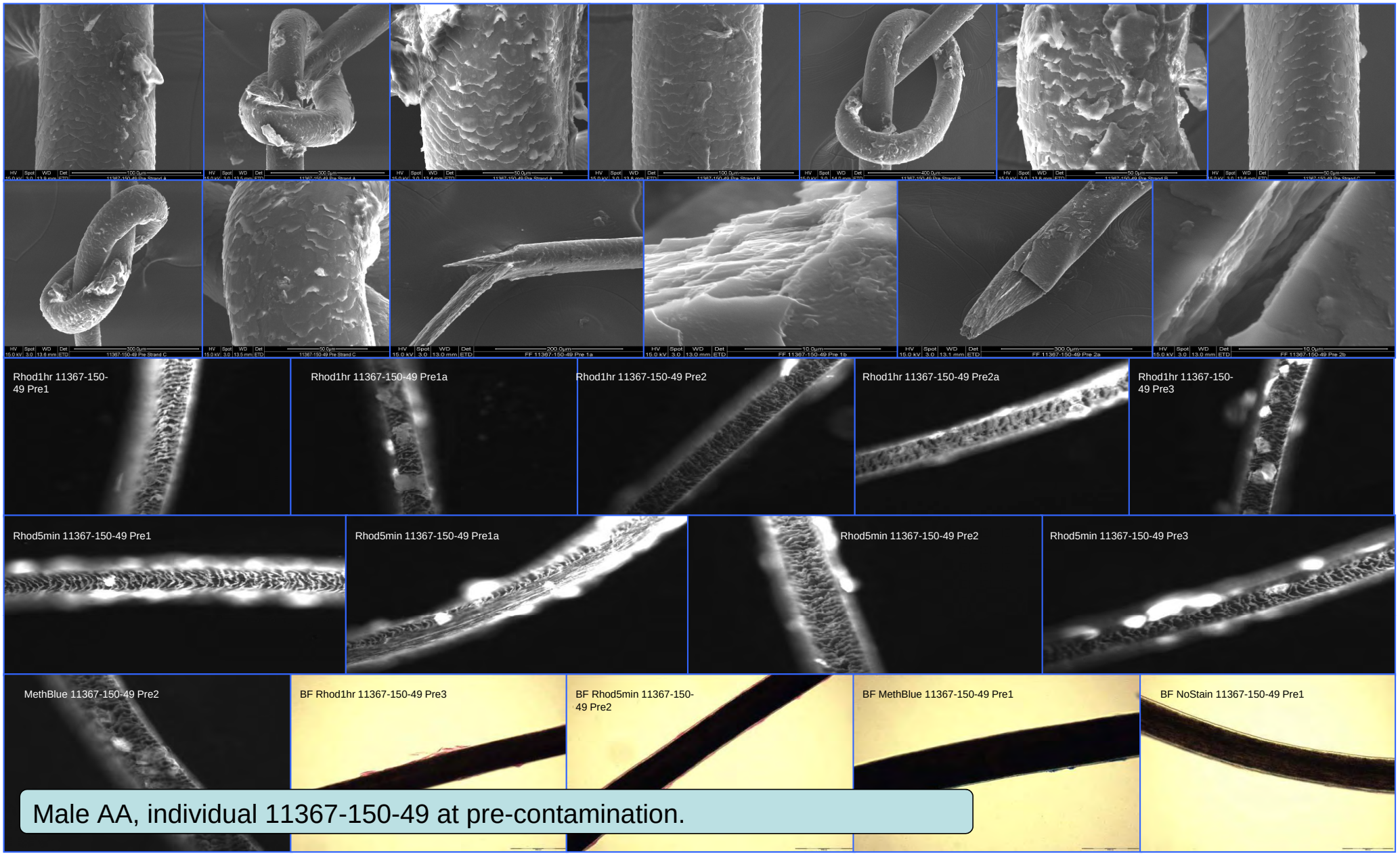

Rhod1hr 11367-150-10 Pre1
Rhod1hr 11367-150-10 Pre2
Rhod1hr 11367-150-10 Pre2a
Rhod1hr 11367-150-10 Pre3
Rhod1hr 11367-150-10 Pre3a
Rhod5min 11367-150-10 Pre1
Rhod5min 11367-150-10 Pre2
Rhod5min 11367-150-10 Pre2c
Rhod5min 11367-150-10 Pre2d
Rhod5min 11367-150-10 Pre3
Rhod5min 11367-150-10 Pre3a
Rhod5min 11367-150-10 Pre3b
Rhod5min 11367-150-10 Pre3c
MethBlue 11367-150-10 Pre1
BF Rhod1hr 11367-150-10 Pre3
BF Rhod5min 11367-150-10 Pre1a
BF MethBlue 11367-150-10 Pre2
BF NoStain 11367-150-10 Pre1
100 mm
100 mm
100 mm
100 mm
Female AA, individual 11367-150-10 at pre-contamination.

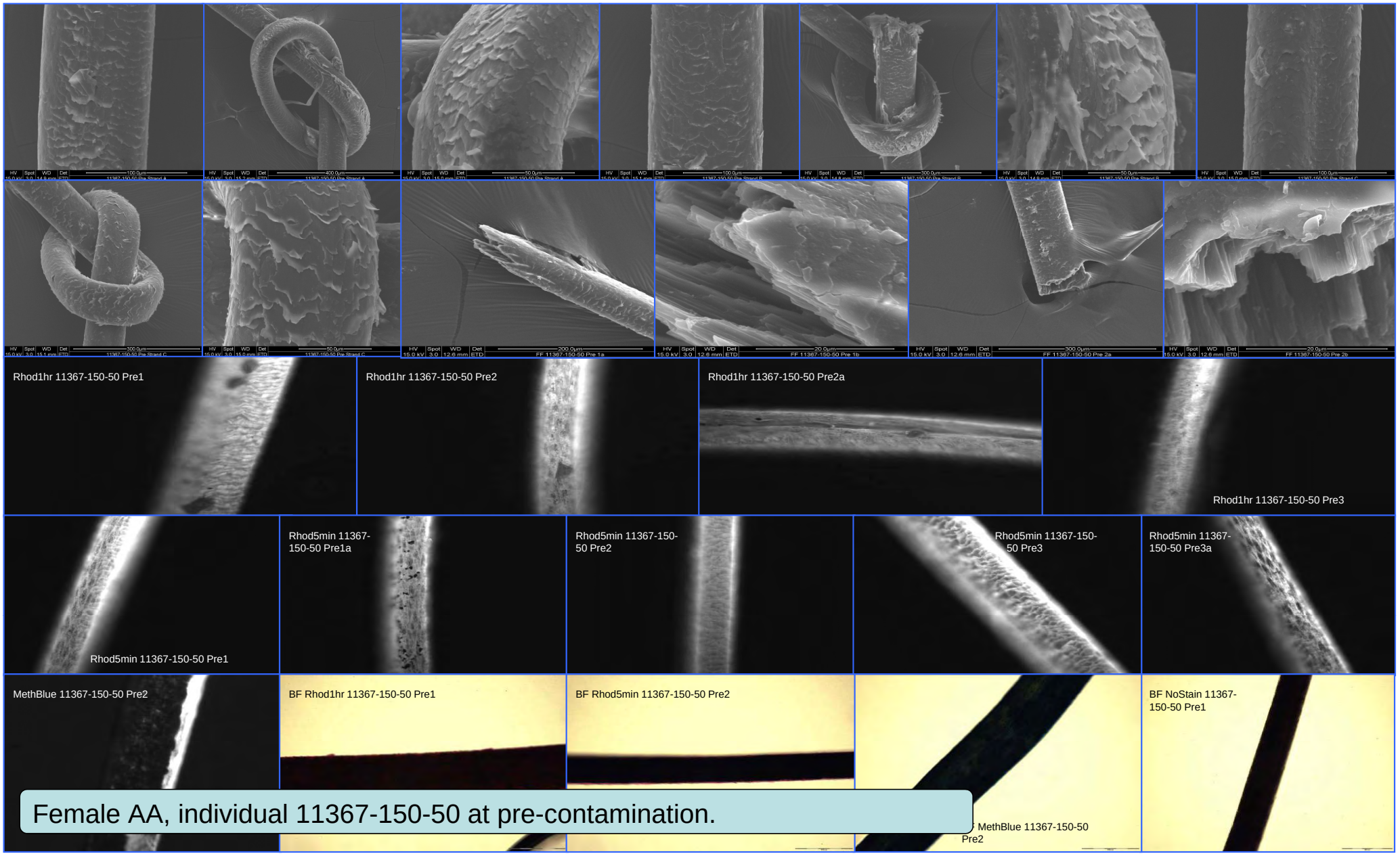

Rhod1hr 11367-150-37 Pre1
Rhod1hr 11367-150-37 Pre1a
Rhod1hr 11367-150-37 Pre2
Rhod1hr 11367-150-37 Pre2a
Rhod1hr 11367-150-37 Pre2b
Rhod1hr 11367-150-37 Pre3
Rhod1hr 11367-150-37 Pre3a
Rhod5min 11367-150-37 Pre1
Rhod5min 11367-150-37 Pre1a
Rhod5min 11367-150-37 Pre2
Rhod5min 11367-150-37 Pre2a
Rhod5min 11367-150-37 Pre3
Rhod5min 11367-150-37 Pre3a
MethBlue 11367-150-37 Pre1
BF Rhod1hr 11367-150-37 Pre2
BF Rhod5min 11367-150-37 Pre1
BF MethBlue 11367-150-37 Pre1
BF NoStain 11367-150-37 Pre1
100 mm
100 mm
100 mm
100 mm
Male AA, individual 11367-150-37 at pre-contamination.

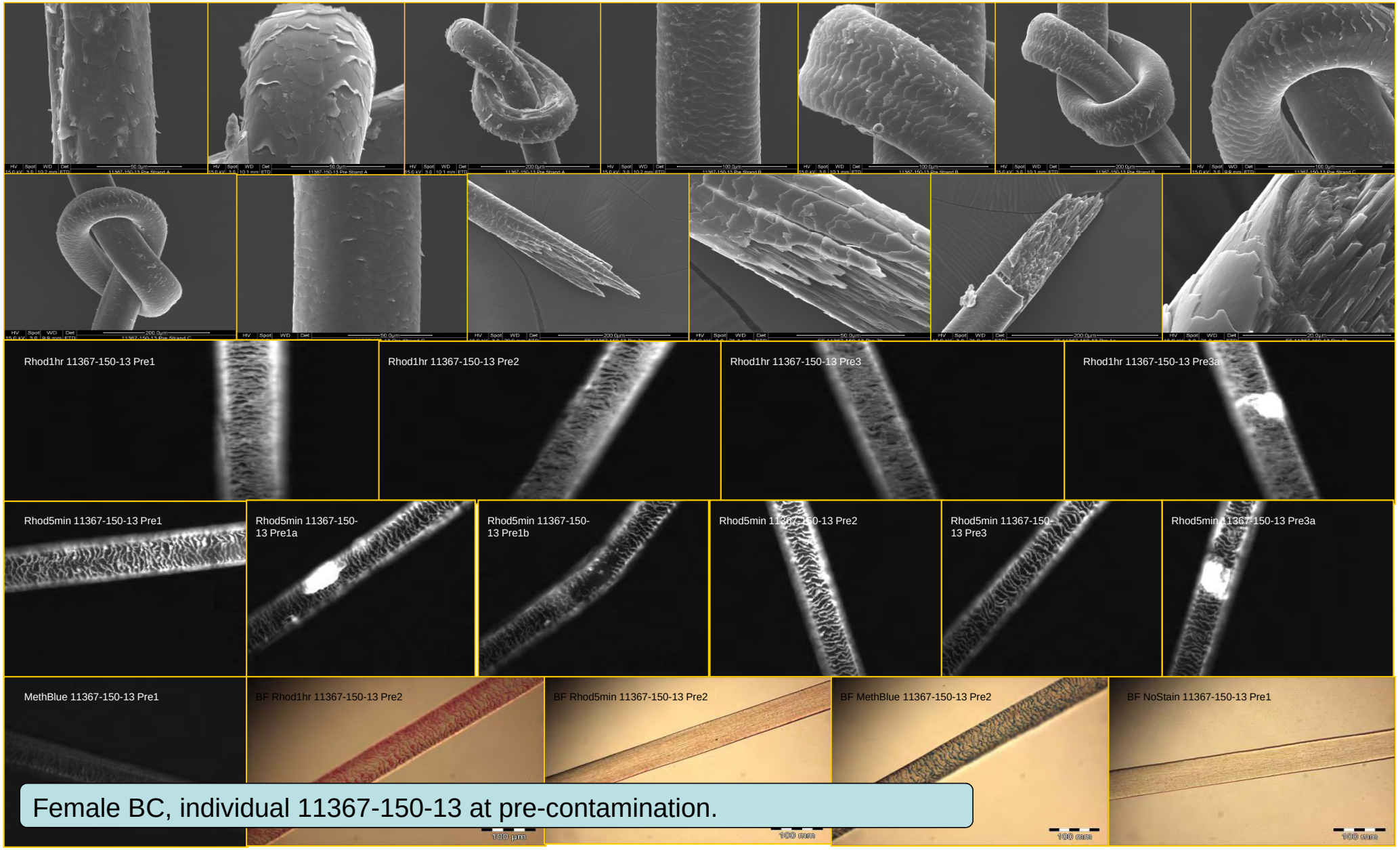

Rhod1hr 11367-150-43 Pre1
Rhod1hr 11367-150-43 Pre1a
Rhod1hr 11367-150-43 Pre2
Rhod1hr 11367-150-43 Pre2a
Rhod1hr 11367-150-43 Pre3
Rhod5min 11367-150-43 Pre1
Rhod5min 11367-150-43 Pre1a
Rhod5min 11367-150-43 Pre2
Rhod5min 11367-150-43 Pre3
Rhod5min 11367-150-43 Pre3a
Rhod5min 11367-150-43 Pre3b
MethBlue 11367-150-43 Pre3
BF Rhod1hr 11367-150-43 Pre2
BF Rhod5min 11367-150-43 Pre1
BF MethBlue 11367-150-43 Pre2
BF NoStain 11367-150-43 Pre1
100 mm
Female AA, individual 11367-150-43 at pre-contamination.

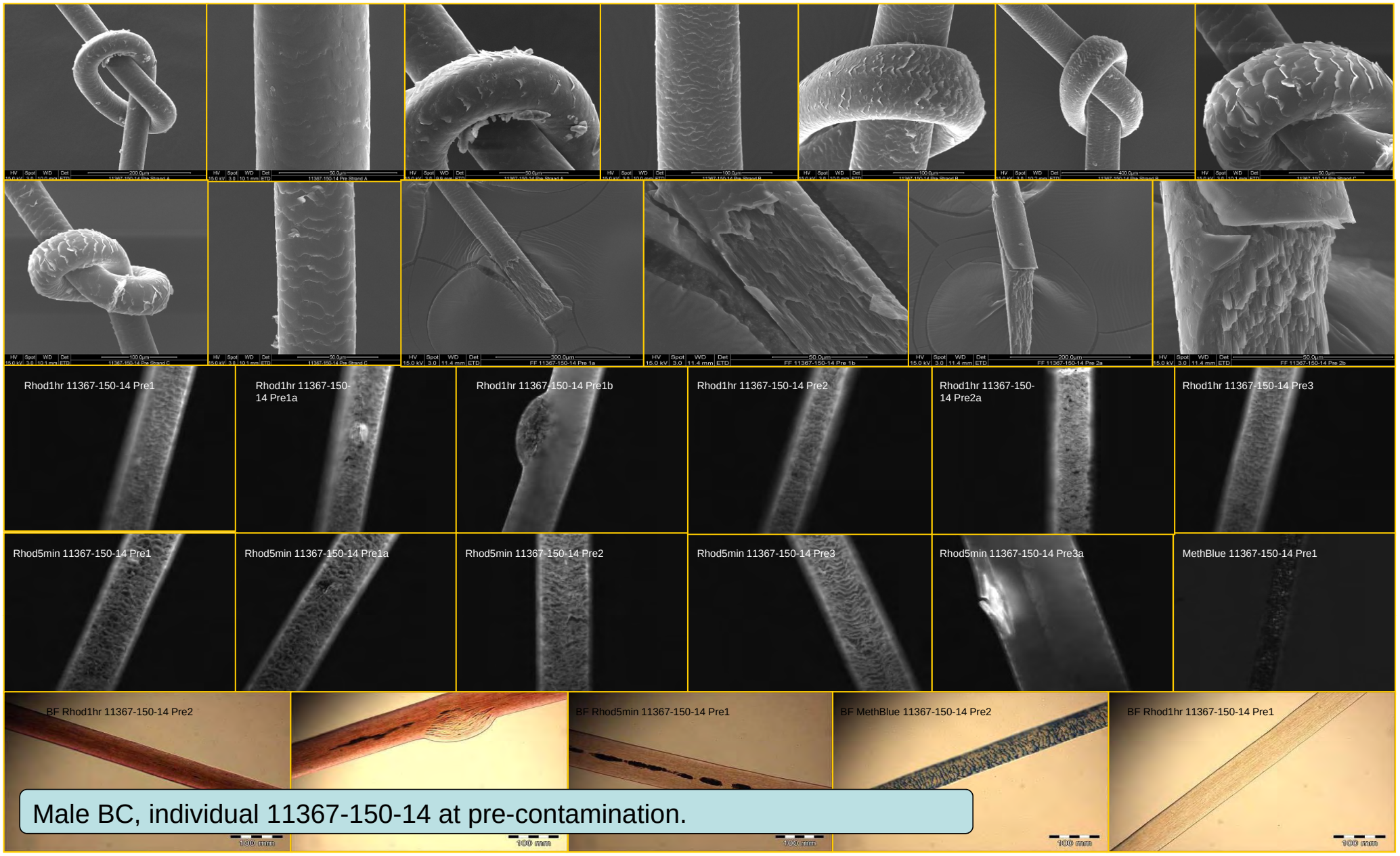

Rhod1hr 11367-150-45 Pre1
Rhod1hr 11367-150-45 Pre2
Rhod1hr 11367-150-45 Pre3
Rhod1hr 11367-150-45 Pre3a
Rhod5min 11367-150-45 Pre1
Rhod5min 11367-150-45 Pre2
Rhod5min 11367-150-45 Pre2a
Rhod5min 11367-150-45 Pre3
Rhod5min 11367-150-45 Pre3a
MethBlue 11367-150-45 Pre1
BF Rhod1hr 11367-150-45 Pre3
BF Rhod5min 11367-150-45 Pre3
BF MethBlue 11367-150-45 Pre2
BF NoStain 11367-150-45 Pre1
100 mm
100 mm
100 mm
100 mm
Female AA, individual 11367-150-45 at pre-contamination.

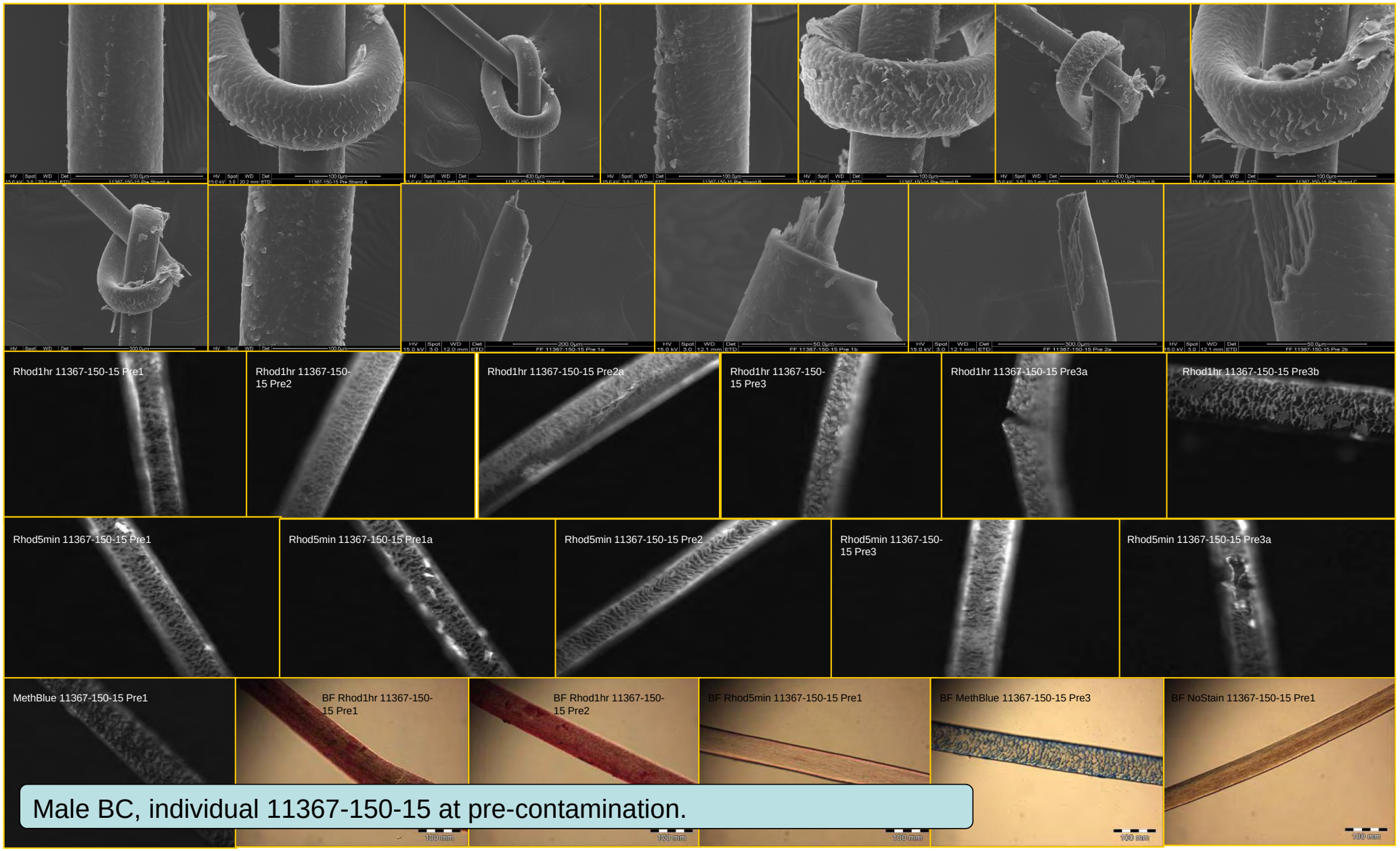

Rhod1hr 11367-150-47 Pre1
Rhod1hr 11367-150-47 Pre1a
Rhod1hr 11367-150-47 Pre2
Rhod1hr 11367-150-47 Pre2a
Rhod1hr 11367-150-47 Pre3
Rhod5min 11367-150-47 Pre1
Rhod5min 11367-150-47 Pre2
Rhod5min 11367-150-47 Pre2a
Rhod5min 11367-150-47 Pre3
Rhod5min 11367-150-47 Pre3a
MethBlue 11367-150-47 Pre3
BF Rhod5min 11367-150-47 Pre1
BF MethBlue 11367-150-47 Pre1
BF NoStain 11367-15047 Pre1
Male AA, individual 11367-150-47 at pre-contamination.

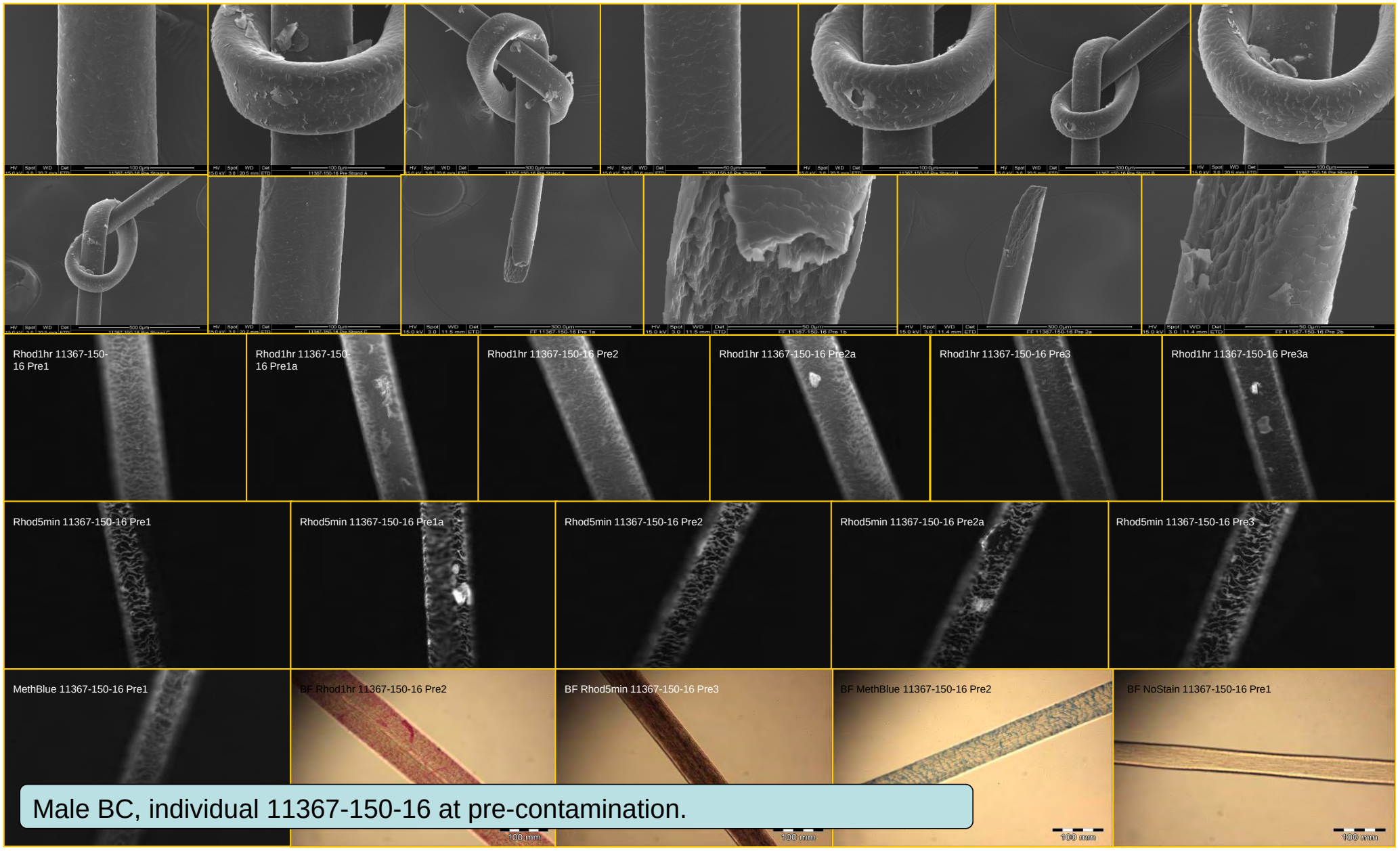

Rhod1hr 11367-150-49 Pre1
Rhod1hr 11367-150-49 Pre1a
Rhod1hr 11367-150-49 Pre2
Rhod1hr 11367-150-49 Pre2a
Rhod1hr 11367-150-49 Pre3
Rhod5min 11367-150-49 Pre1
Rhod5min 11367-150-49 Pre1a
Rhod5min 11367-150-49 Pre2
Rhod5min 11367-150-49 Pre3
MethBlue 11367-150-49 Pre2
BF Rhod1hr 11367-150-49 Pre3
BF Rhod5min 11367-150-49 Pre2
BF MethBlue 11367-150-49 Pre1
BF NoStain 11367-150-49 Pre1
Male AA, individual 11367-150-49 at pre-contamination.

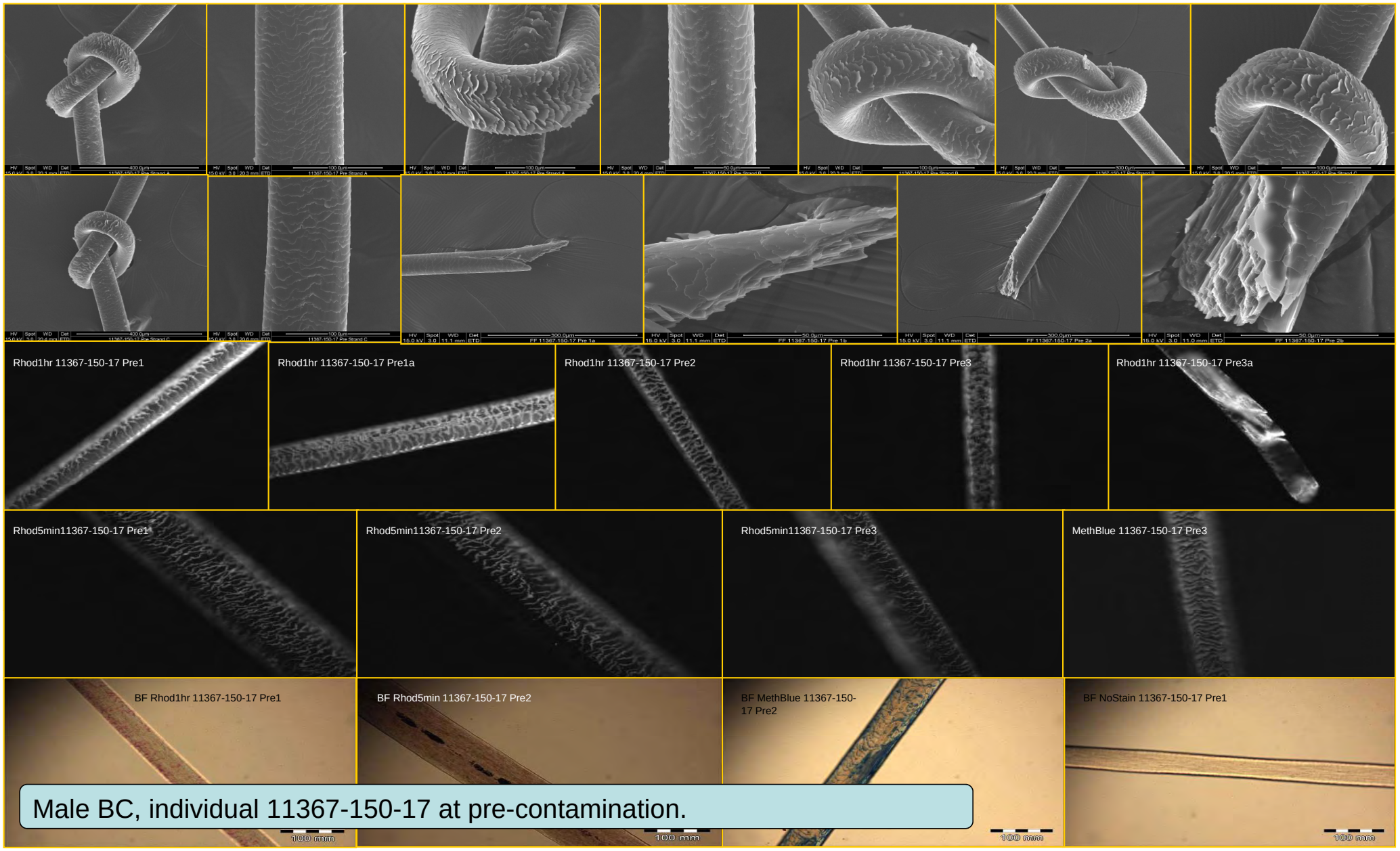

Rhod1hr 11367-150-50 Pre1
Rhod1hr 11367-150-50 Pre2
Rhod1hr 11367-150-50 Pre2a
Rhod1hr 11367-150-50 Pre3
Rhod5min 11367-150-50 Pre1
Rhod5min 11367-150-50 Pre1a
Rhod5min 11367-150-50 Pre2
Rhod5min 11367-150-50 Pre3
Rhod5min 11367-150-50 Pre3a
MethBlue 11367-150-50 Pre2
BF Rhod1hr 11367-150-50 Pre1
BF Rhod5min 11367-150-50 Pre2
BF NoStain 11367-150-50 Pre1
Female AA, individual 11367-150-50 at pre-contamination.
MethBlue 11367-150-50 Pre2

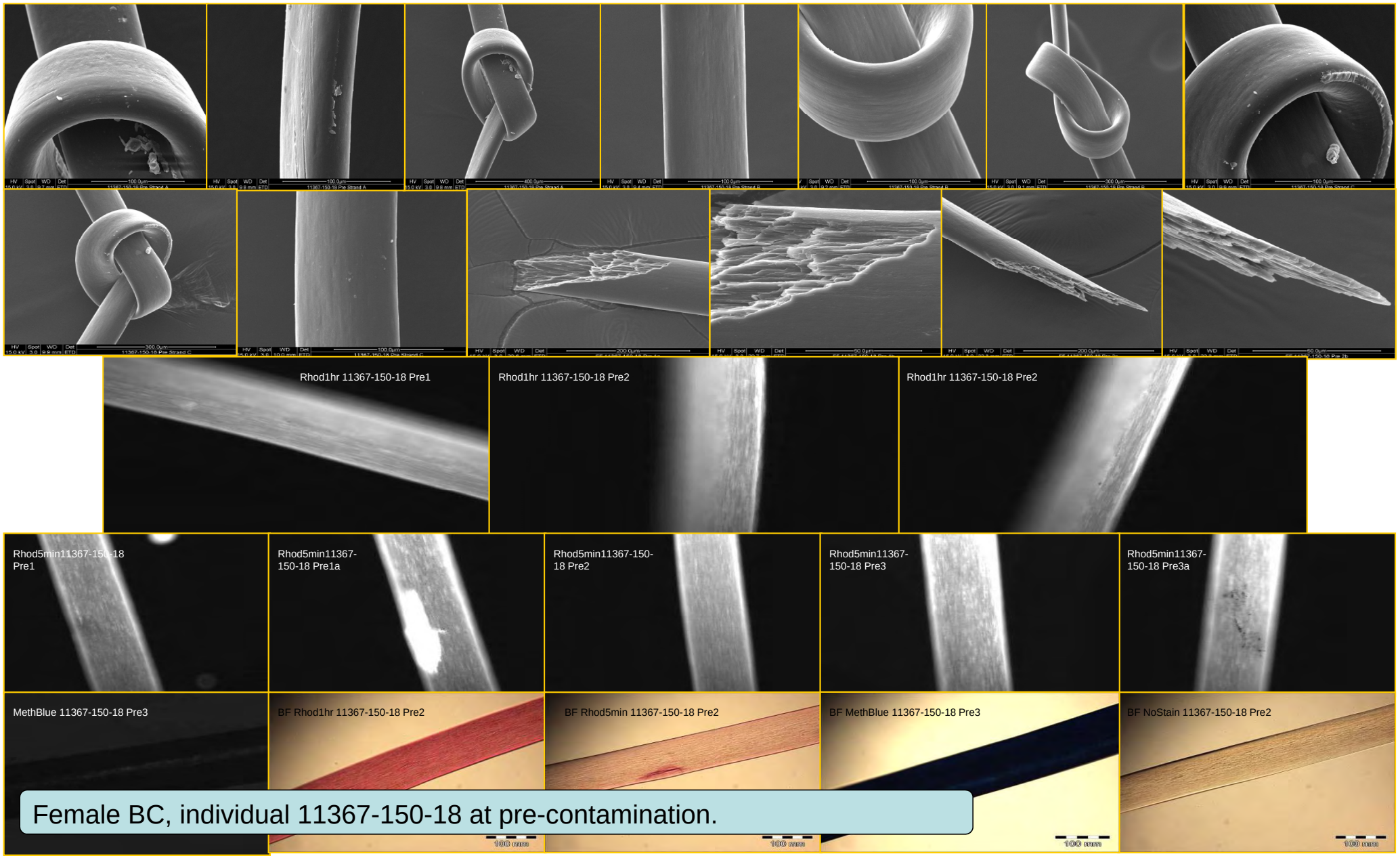

Rhod1hr 11367-150-13 Pre1
Rhod1hr 11367-150-13 Pre2
Rhod1hr 11367-150-13 Pre3
Rhod1hr 11367-150-13 Pre3a
Rhod5min 11367-150-13 Pre1
Rhod5min 11367-150-13 Pre1a
Rhod5min 11367-150-13 Pre1b
Rhod5min 11367-150-13 Pre2
Rhod5min 11367-150-13 Pre3
Rhod5min 11367-150-13 Pre3a
MethBlue 11367-150-13 Pre1
BF Rhod1hr 11367-150-13 Pre2
BF Rhod5min 11367-150-13 Pre2
BF MethBlue 11367-150-13 Pre2
BF NoStain 11367-150-13 Pre1
100 µm
100 mm
100 mm
100 mm
Female BC, individual 11367-150-13 at pre-contamination.


Rhod1hr 11367-150-14 Pre1
Rhod1hr 11367-150-14 Pre1a
Rhod1hr 11367-150-14 Pre1b
Rhod1hr 11367-150-14 Pre2
Rhod1hr 11367-150-14 Pre2a
Rhod1hr 11367-150-14 Pre3
Rhod5min 11367-150-14 Pre1
Rhod5min 11367-150-14 Pre1a
Rhod5min 11367-150-14 Pre2
Rhod5min 11367-150-14 Pre3
Rhod5min 11367-150-14 Pre3a
MethBlue 11367-150-14 Pre1
BF Rhod1hr 11367-150-14 Pre2
BF Rhod5min 11367-150-14 Pre1
BF MethBlue 11367-150-14 Pre2
BF Rhod1hr 11367-150-14 Pre1
Male BC, individual 11367-150-14 at pre-contamination.

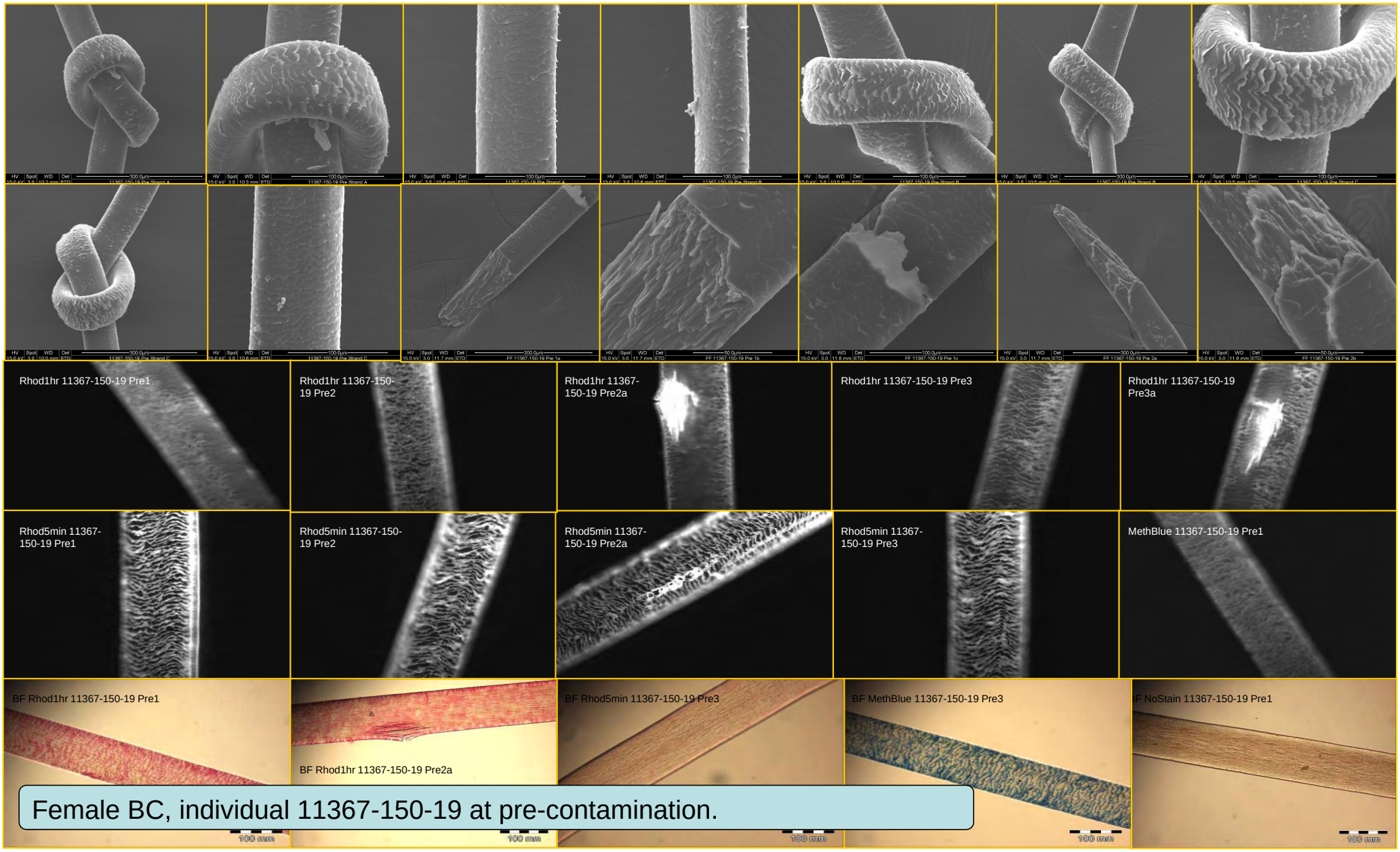

Rhod1hr 11367-150-15 Pre1
Rhod1hr 11367-150-15 Pre2
Rhod1hr 11367-150-15 Pre2a
Rhod1hr 11367-150-15 Pre3
Rhod1hr 11367-150-15 Pre3a
Rhod1hr 11367-150-15 Pre3b
Rhod5min 11367-150-15 Pre1
Rhod5min 11367-150-15 Pre1a
Rhod5min 11367-150-15 Pre2
Rhod5min 11367-150-15 Pre3
Rhod5min 11367-150-15 Pre3a
MethBlue 11367-150-15 Pre1
BF Rhod1hr 11367-150-15 Pre1
BF Rhod1hr 11367-150-15 Pre2
BF Rhod5min 11367-150-15 Pre1
BF MethBlue 11367-150-15 Pre3
BF NoStain 11367-150-15 Pre1
Male BC, individual 11367-150-15 at pre-contamination.


Rhod1hr 11367-150-16 Pre1
Rhod1hr 11367-150-16 Pre1a
Rhod1hr 11367-150-16 Pre2
Rhod1hr 11367-150-16 Pre2a
Rhod1hr 11367-150-16 Pre3
Rhod1hr 11367-150-16 Pre3a
Rhod5min 11367-150-16 Pre1
Rhod5min 11367-150-16 Pre1a
Rhod5min 11367-150-16 Pre2
Rhod5min 11367-150-16 Pre2a
Rhod5min 11367-150-16 Pre3
MethBlue 11367-150-16 Pre1
BF Rhod1hr 11367-150-16 Pre2
BF Rhod5min 11367-150-16 Pre3
BF MethBlue 11367-150-16 Pre2
BF NoStain 11367-150-16 Pre1
100 mm
Male BC, individual 11367-150-16 at pre-contamination.

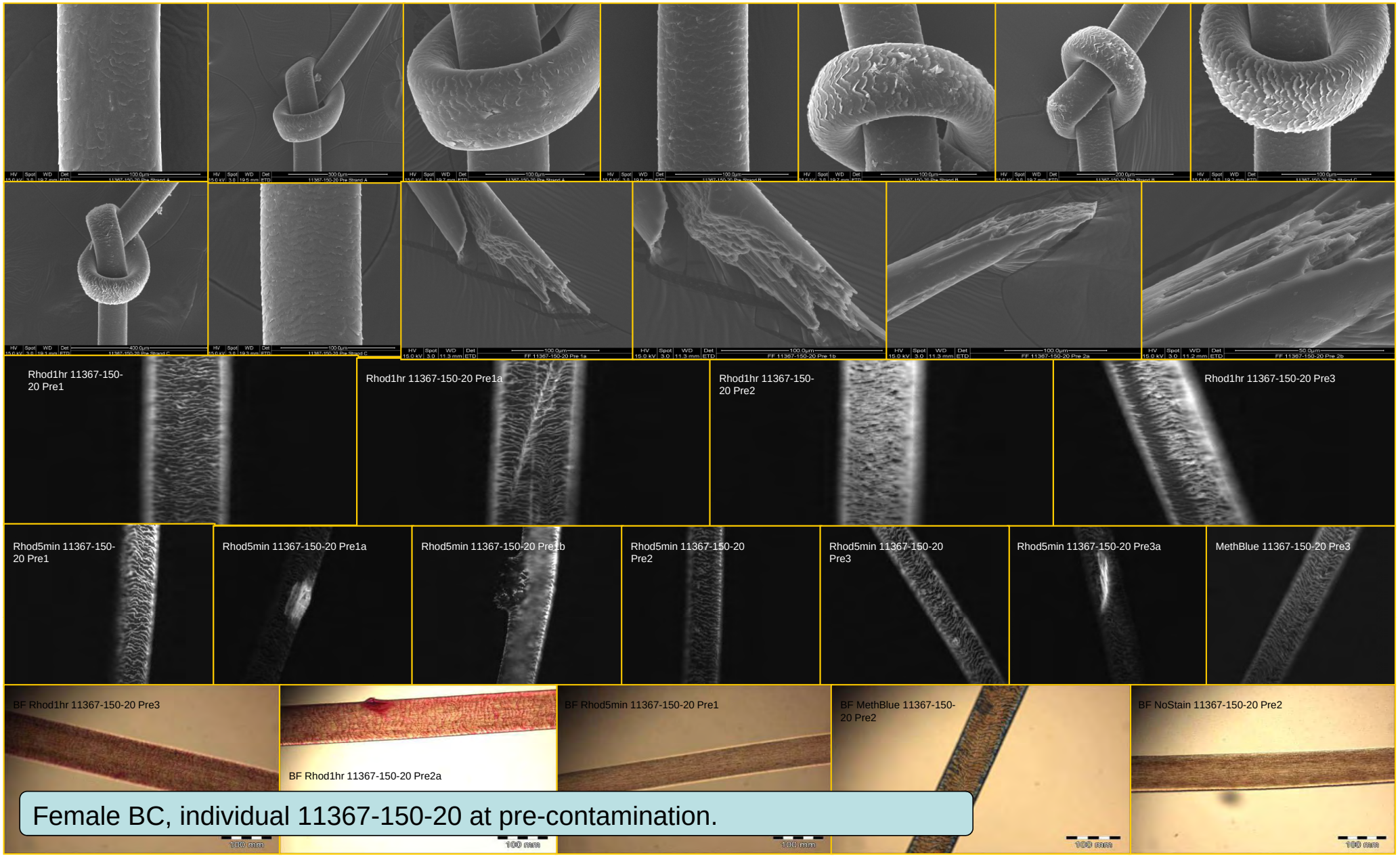

Rhod1hr 11367-150-17 Pre1
Rhod1hr 11367-150-17 Pre1a
Rhod1hr 11367-150-17 Pre2
Rhod1hr 11367-150-17 Pre3
Rhod1hr 11367-150-17 Pre3a
Rhod5min11367-150-17 Pre1
Rhod5min11367-150-17 Pre2
Rhod5min11367-150-17 Pre3
MethBlue 11367-150-17 Pre3
BF Rhod1hr 11367-150-17 Pre1
BF Rhod5min 11367-150-17 Pre2
BF MethBlue 11367-150-17 Pre2
BF NoStain 11367-150-17 Pre1
100 mm
Male BC, individual 11367-150-17 at pre-contamination.

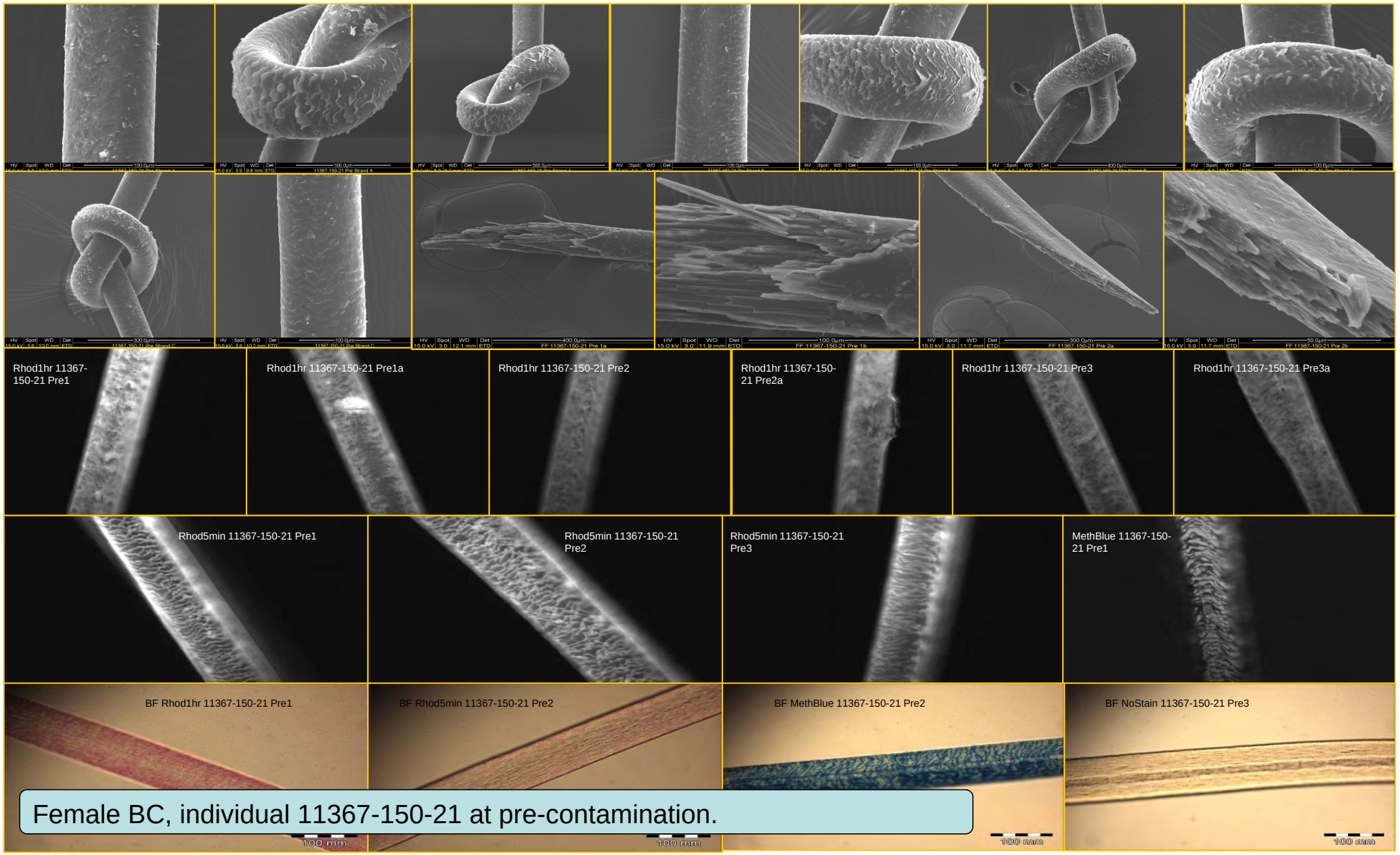


Female BC, individual 11367-150-18 at pre-contamination.

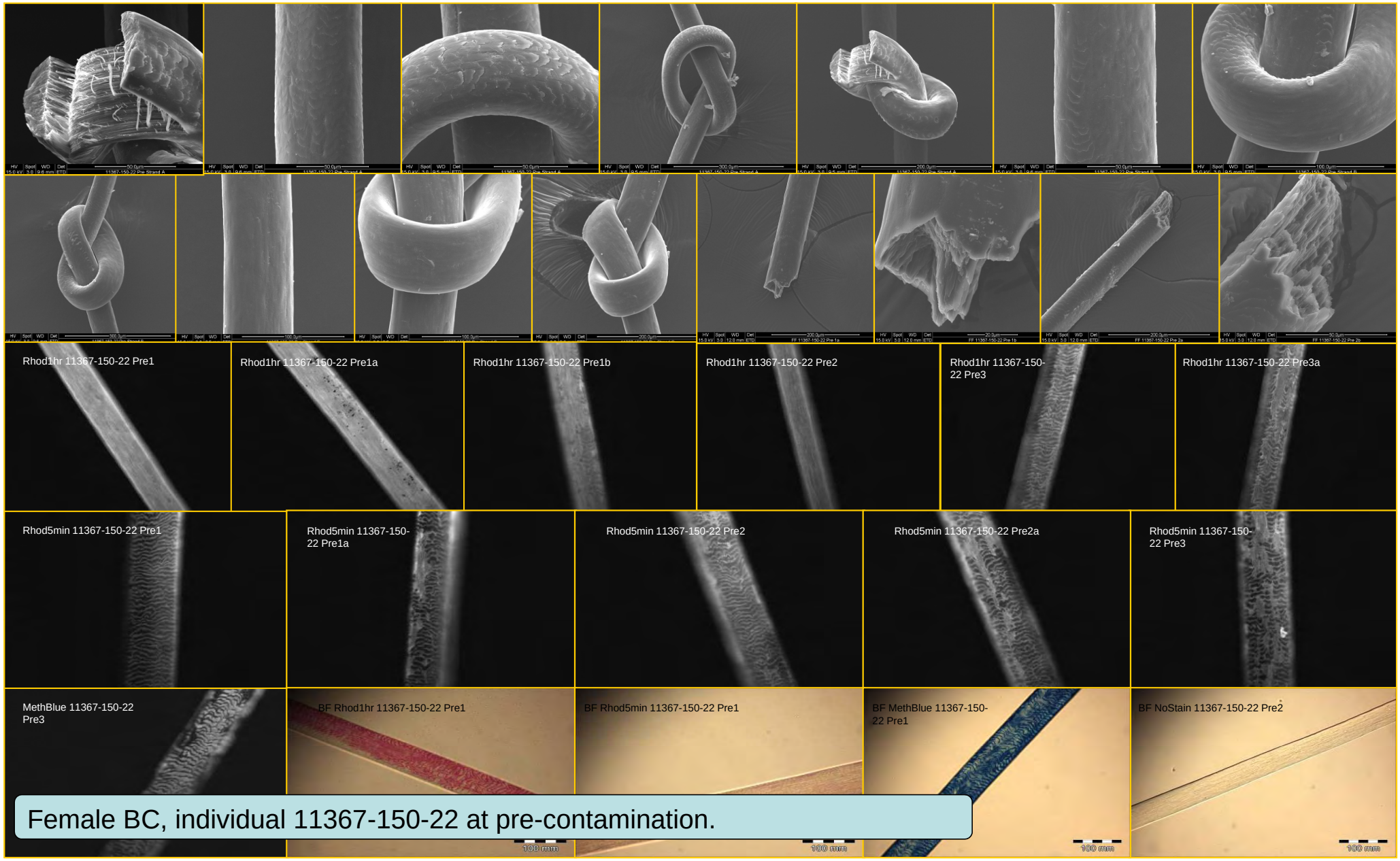


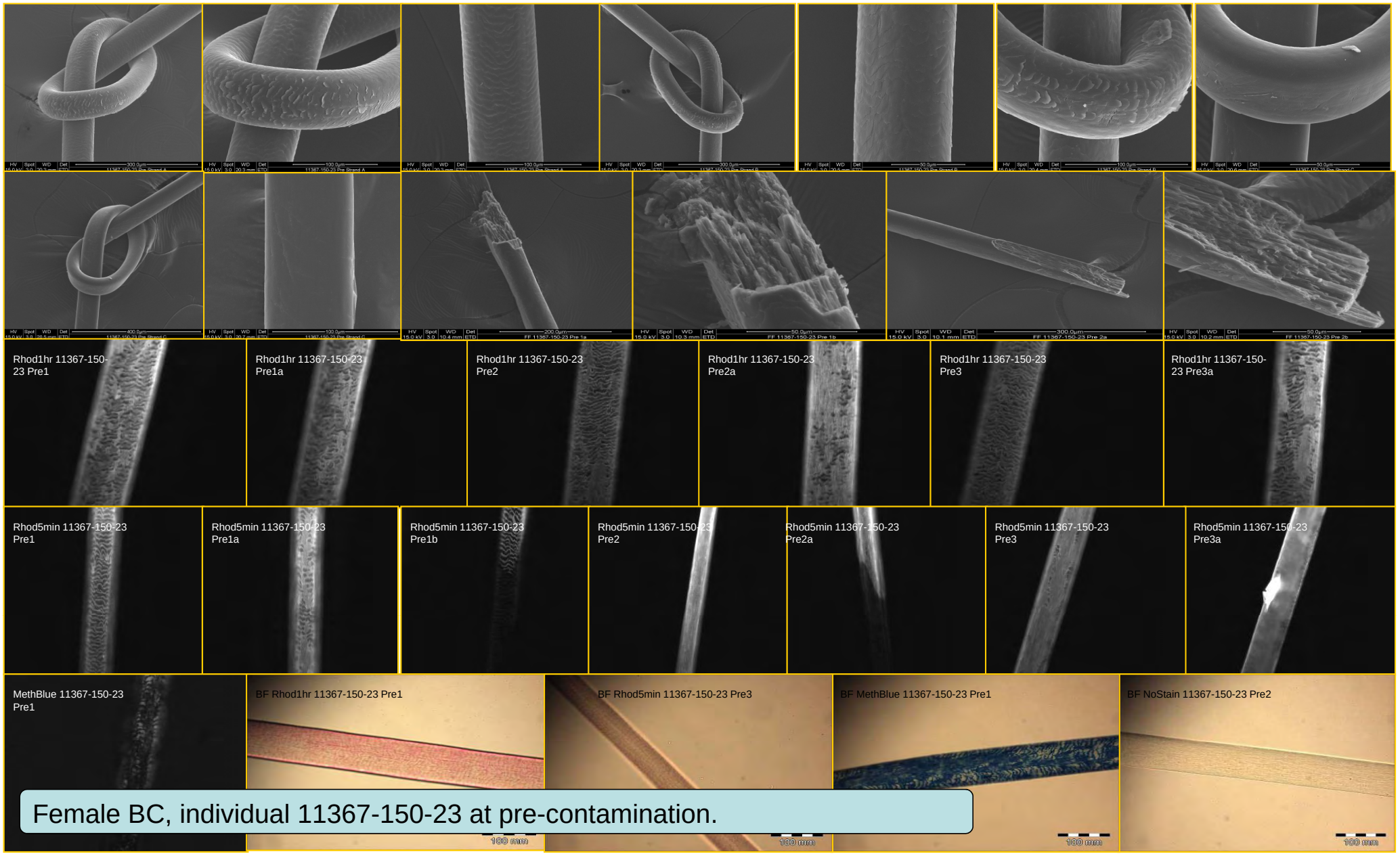
Female BC, individual 11367-150-19 at pre-contamination.

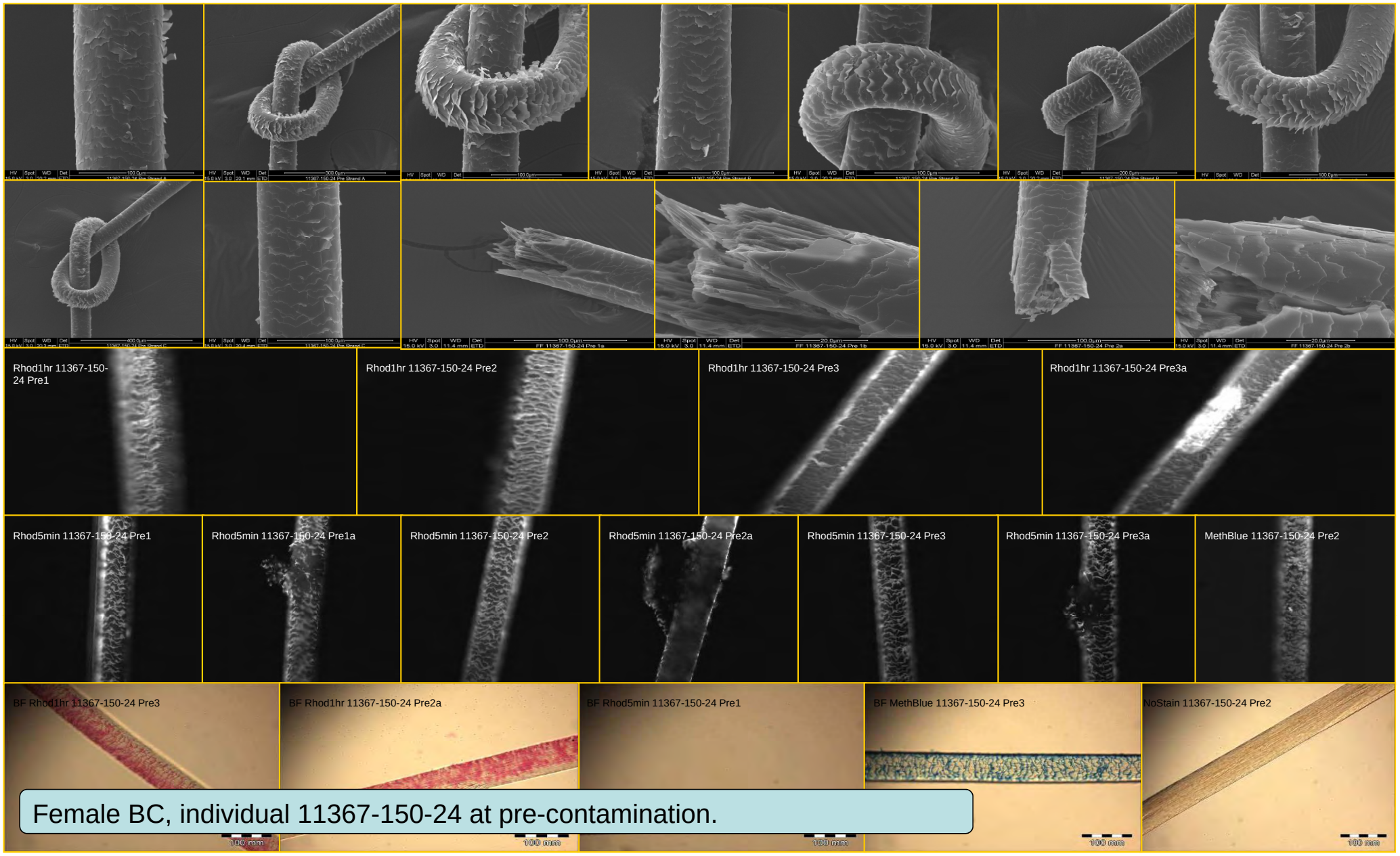

Rhod1hr 11367-150-20 Pre1
Rhod1hr 11367-150-20 Pre1a
Rhod1hr 11367-150-20 Pre2
Rhod1hr 11367-150-20 Pre3
Rhod5min 11367-150-20 Pre1
Rhod5min 11367-150-20 Pre1a
Rhod5min 11367-150-20 Pre1b
Rhod5min 11367-150-20 Pre2
Rhod5min 11367-150-20 Pre3
Rhod5min 11367-150-20 Pre3a
MethBlue 11367-150-20 Pre3
BF Rhod1hr 11367-150-20 Pre3
BF Rhod1hr 11367-150-20 Pre2a
BF Rhod5min 11367-150-20 Pre1
BF MethBlue 11367-150-20 Pre2
BF NoStain 11367-150-20 Pre2
Female BC, individual 11367-150-20 at pre-contamination.

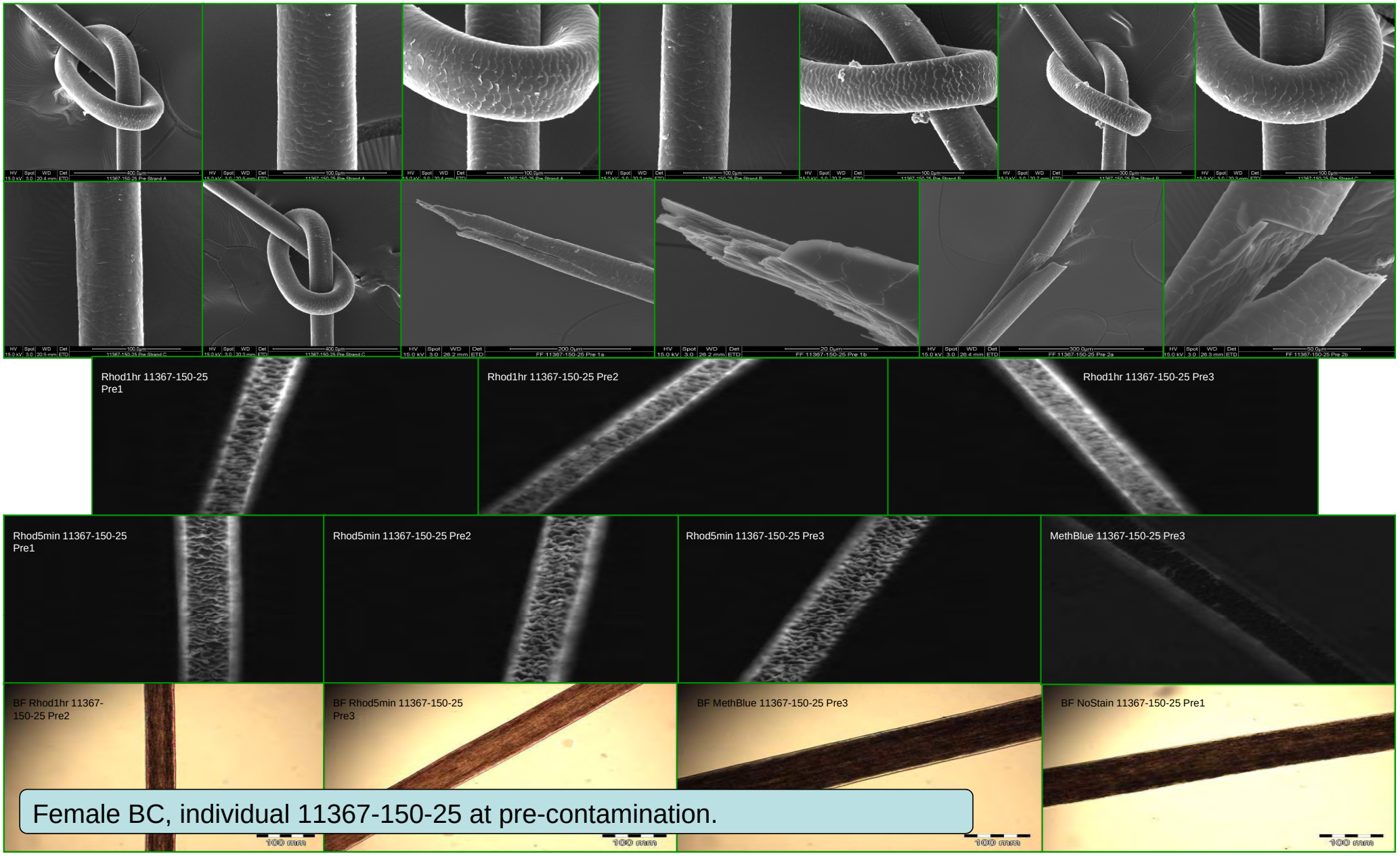


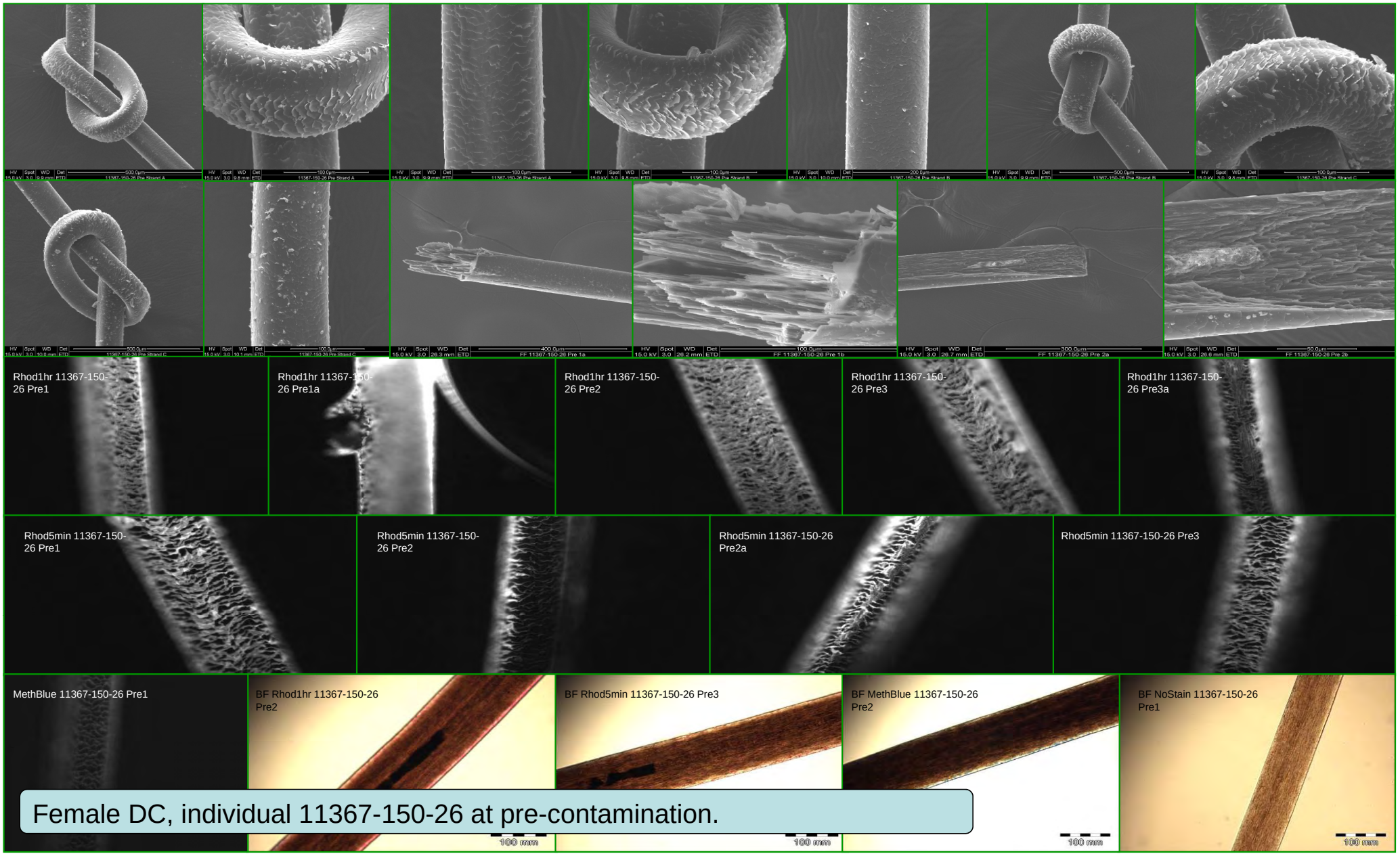
Female BC, individual 11367-150-21 at pre-contamination.

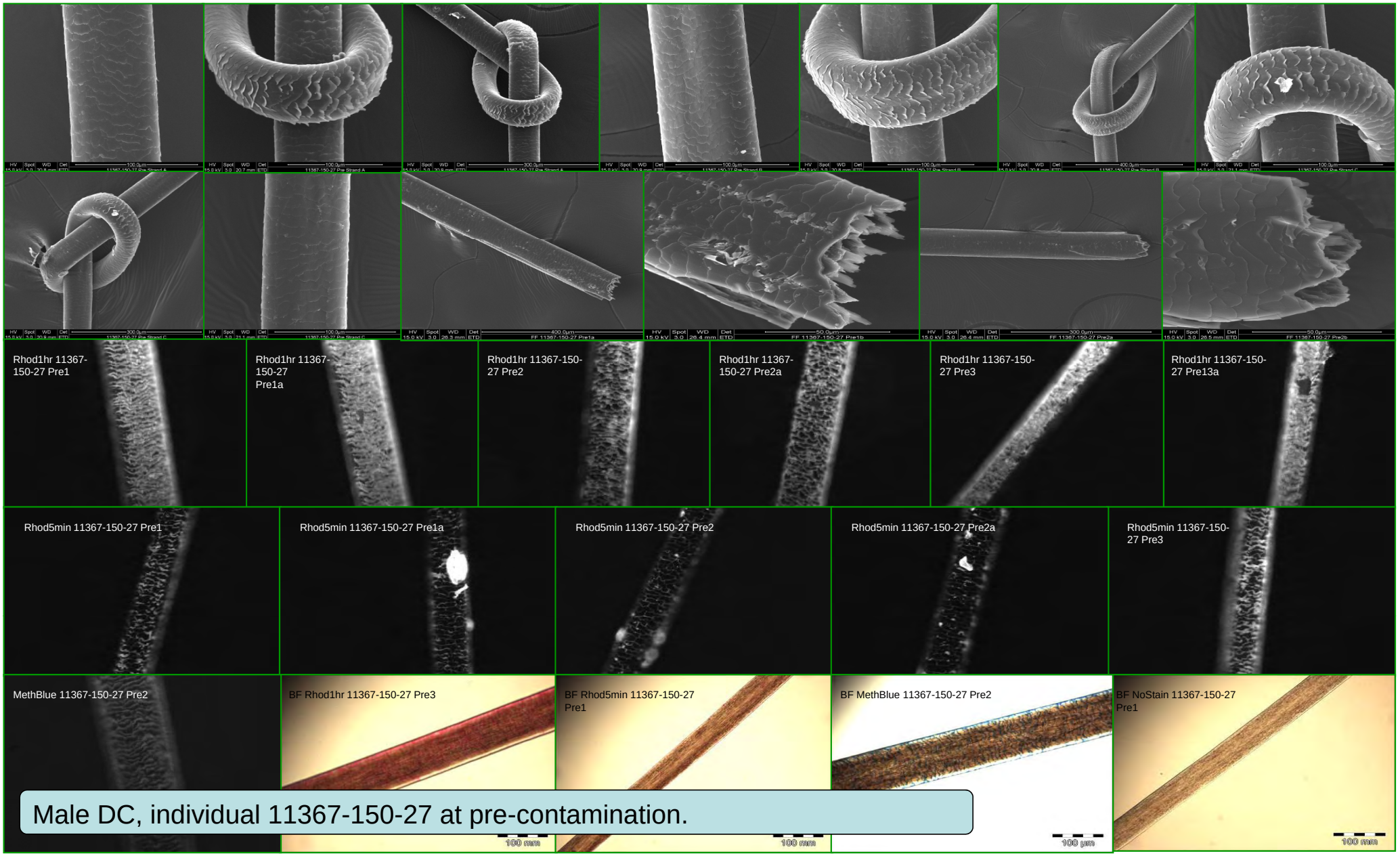

Rhod1hr 11367-150-22 Pre1
Rhod1hr 11367-150-22 Pre1a
Rhod1hr 11367-150-22 Pre1b
Rhod1hr 11367-150-22 Pre2
Rhod1hr 11367-150-22 Pre3
Rhod1hr 11367-150-22 Pre3a
Rhod5min 11367-150-22 Pre1
Rhod5min 11367-150-22 Pre1a
Rhod5min 11367-150-22 Pre2
Rhod5min 11367-150-22 Pre2a
Rhod5min 11367-150-22 Pre3
MethBlue 11367-150-22 Pre3
BF Rhod1hr 11367-150-22 Pre1
BF Rhod5min 11367-150-22 Pre1
BF MethBlue 11367-150-22 Pre1
BF NoStain 11367-150-22 Pre2
100 mm
Female BC, individual 11367-150-22 at pre-contamination.

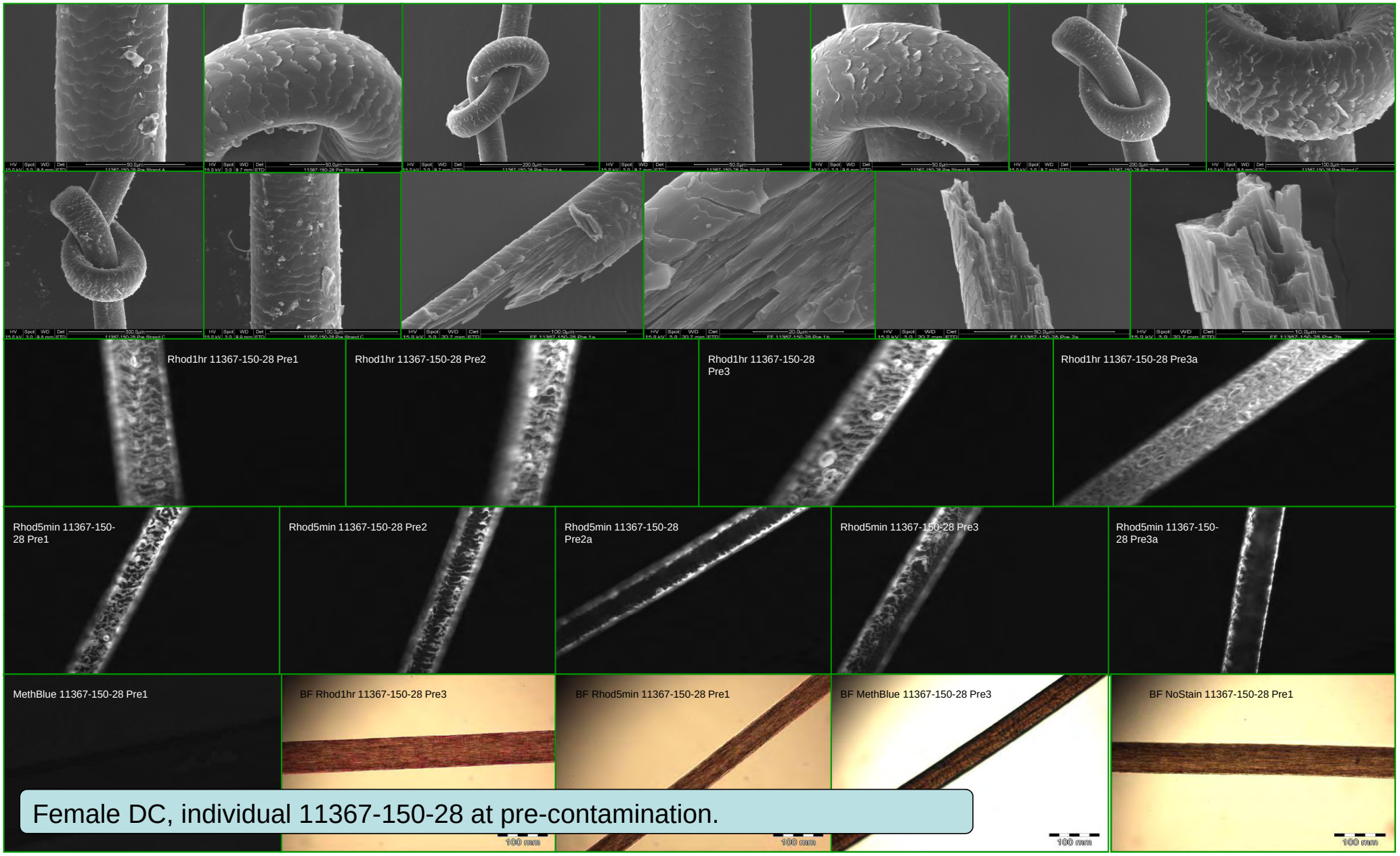

Rhod1hr 11367-150-23 Pre1
Rhod1hr 11367-150-23 Pre1a
Rhod1hr 11367-150-23 Pre2
Rhod1hr 11367-150-23 Pre2a
Rhod1hr 11367-150-23 Pre3
Rhod1hr 11367-150-23 Pre3a
Rhod5min 11367-150-23 Pre1
Rhod5min 11367-150-23 Pre1a
Rhod5min 11367-150-23 Pre1b
Rhod5min 11367-150-23 Pre2
Rhod5min 11367-150-23 Pre2a
Rhod5min 11367-150-23 Pre3
Rhod5min 11367-150-23 Pre3a
MethBlue 11367-150-23 Pre1
BF Rhod1hr 11367-150-23 Pre1
BF Rhod5min 11367-150-23 Pre3
BF MethBlue 11367-150-23 Pre1
BF NoStain 11367-150-23 Pre2
100 mm
Female BC, individual 11367-150-23 at pre-contamination.

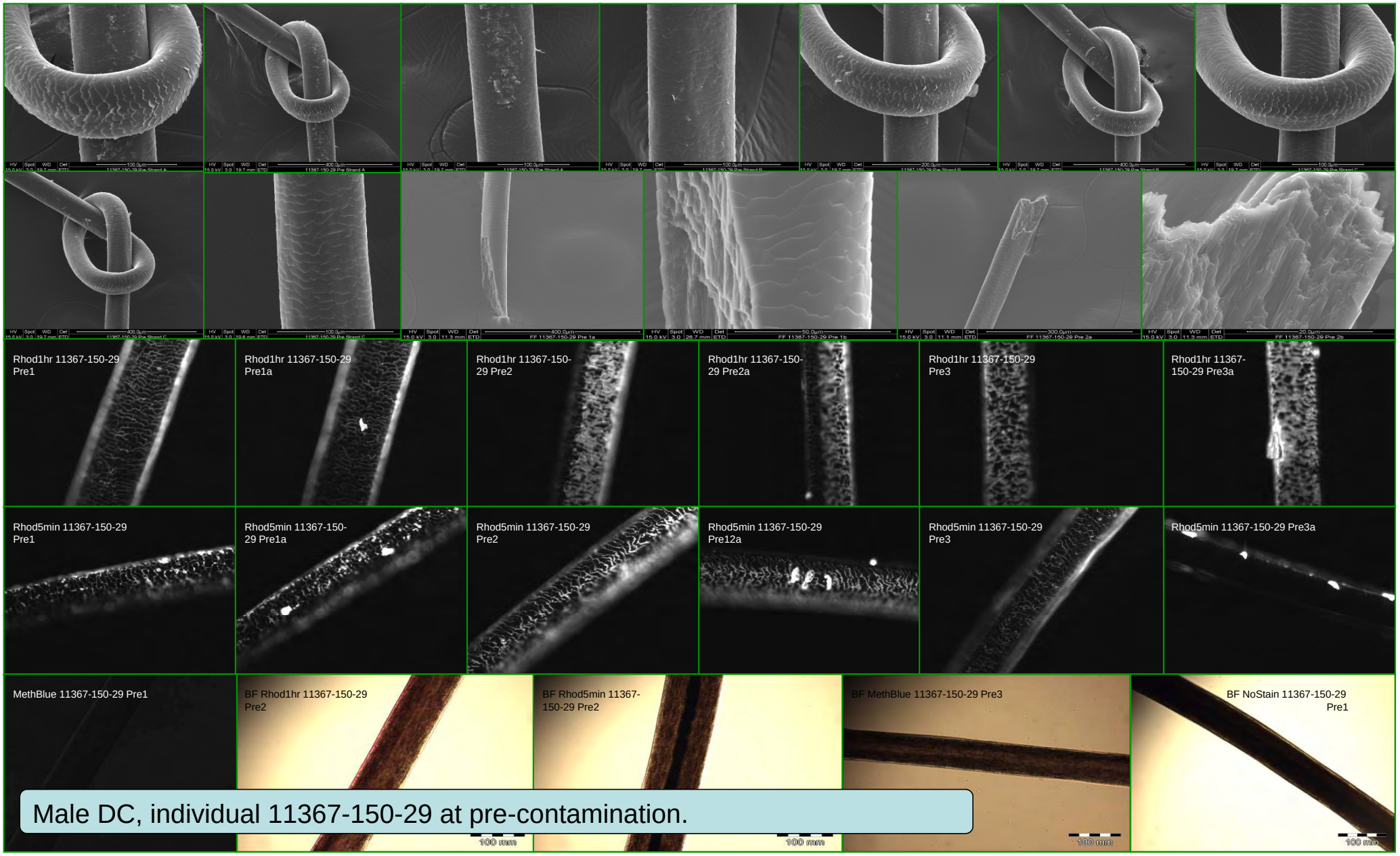

Rhod1hr 11367-150-24 Pre1
Rhod1hr 11367-150-24 Pre2
Rhod1hr 11367-150-24 Pre3
Rhod1hr 11367-150-24 Pre3a
Rhod5min 11367-150-24 Pre1
Rhod5min 11367-150-24 Pre1a
Rhod5min 11367-150-24 Pre2
Rhod5min 11367-150-24 Pre2a
Rhod5min 11367-150-24 Pre3
Rhod5min 11367-150-24 Pre3a
MethBlue 11367-150-24 Pre2
BF Rhod1hr 11367-150-24 Pre3
BF Rhod1hr 11367-150-24 Pre2a
BF Rhod5min 11367-150-24 Pre1
BF MethBlue 11367-150-24 Pre3
NoStain 11367-150-24 Pre2
Female BC, individual 11367-150-24 at pre-contamination.

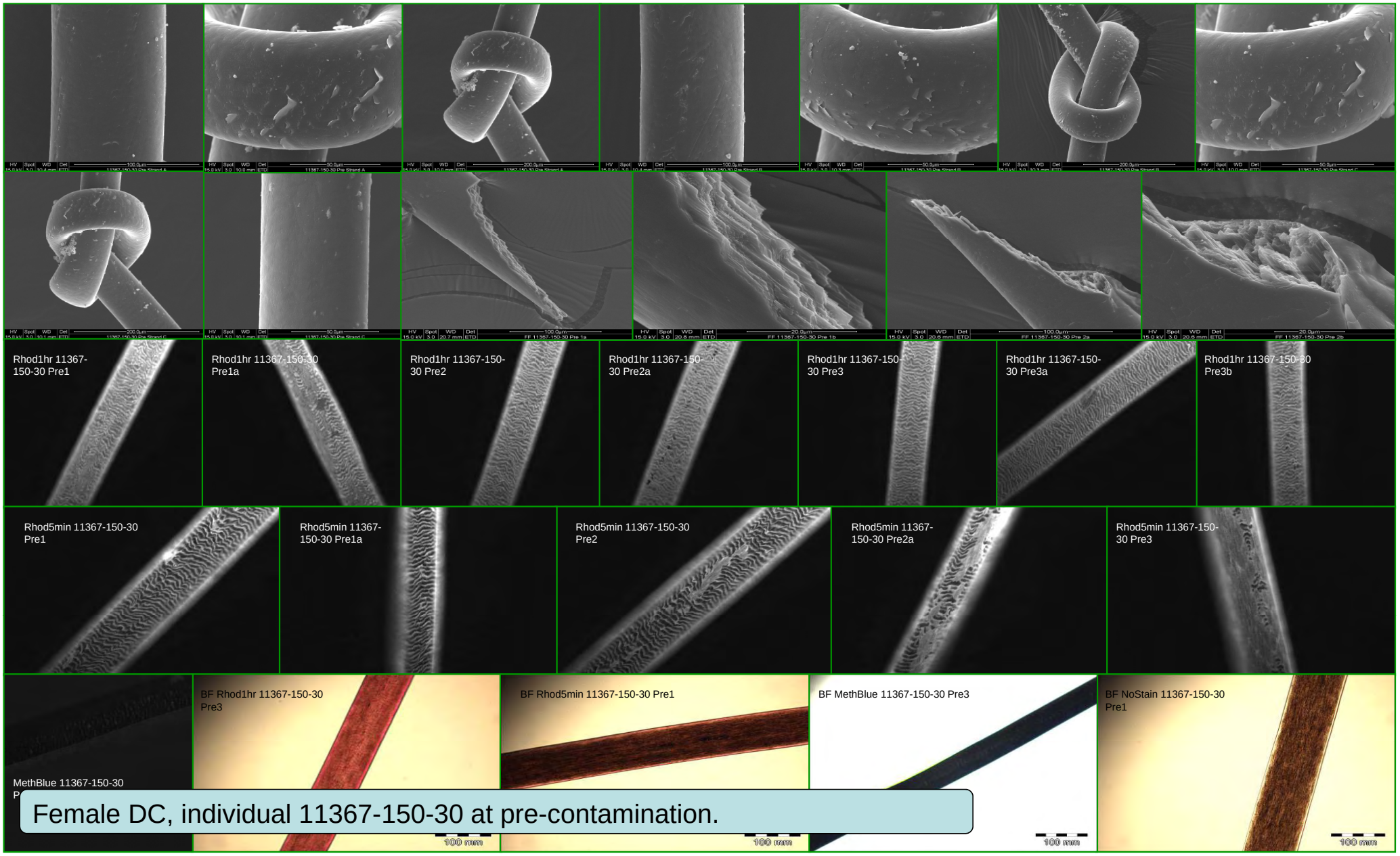

Rhod1hr 11367-150-25 Pre1
Rhod1hr 11367-150-25 Pre2
Rhod1hr 11367-150-25 Pre3
Rhod5min 11367-150-25 Pre1
Rhod5min 11367-150-25 Pre2
Rhod5min 11367-150-25 Pre3
MethBlue 11367-150-25 Pre3
BF Rhod1hr 11367-150-25 Pre2
BF Rhod5min 11367-150-25 Pre3
BF MethBlue 11367-150-25 Pre3
BF NoStain 11367-150-25 Pre1
100 mm
Female BC, individual 11367-150-25 at pre-contamination.


Rhod1hr 11367-150-26 Pre1
Rhod1hr 11367-150-26 Pre1a
Rhod1hr 11367-150-26 Pre2
Rhod1hr 11367-150-26 Pre3
Rhod1hr 11367-150-26 Pre3a
Rhod5min 11367-150-26 Pre1
Rhod5min 11367-150-26 Pre2
Rhod5min 11367-150-26 Pre2a
Rhod5min 11367-150-26 Pre3
MethBlue 11367-150-26 Pre1
BF Rhod1hr 11367-150-26 Pre2
BF Rhod5min 11367-150-26 Pre3
BF MethBlue 11367-150-26 Pre2
BF NoStain 11367-150-26 Pre1
100 mm
Female DC, individual 11367-150-26 at pre-contamination.

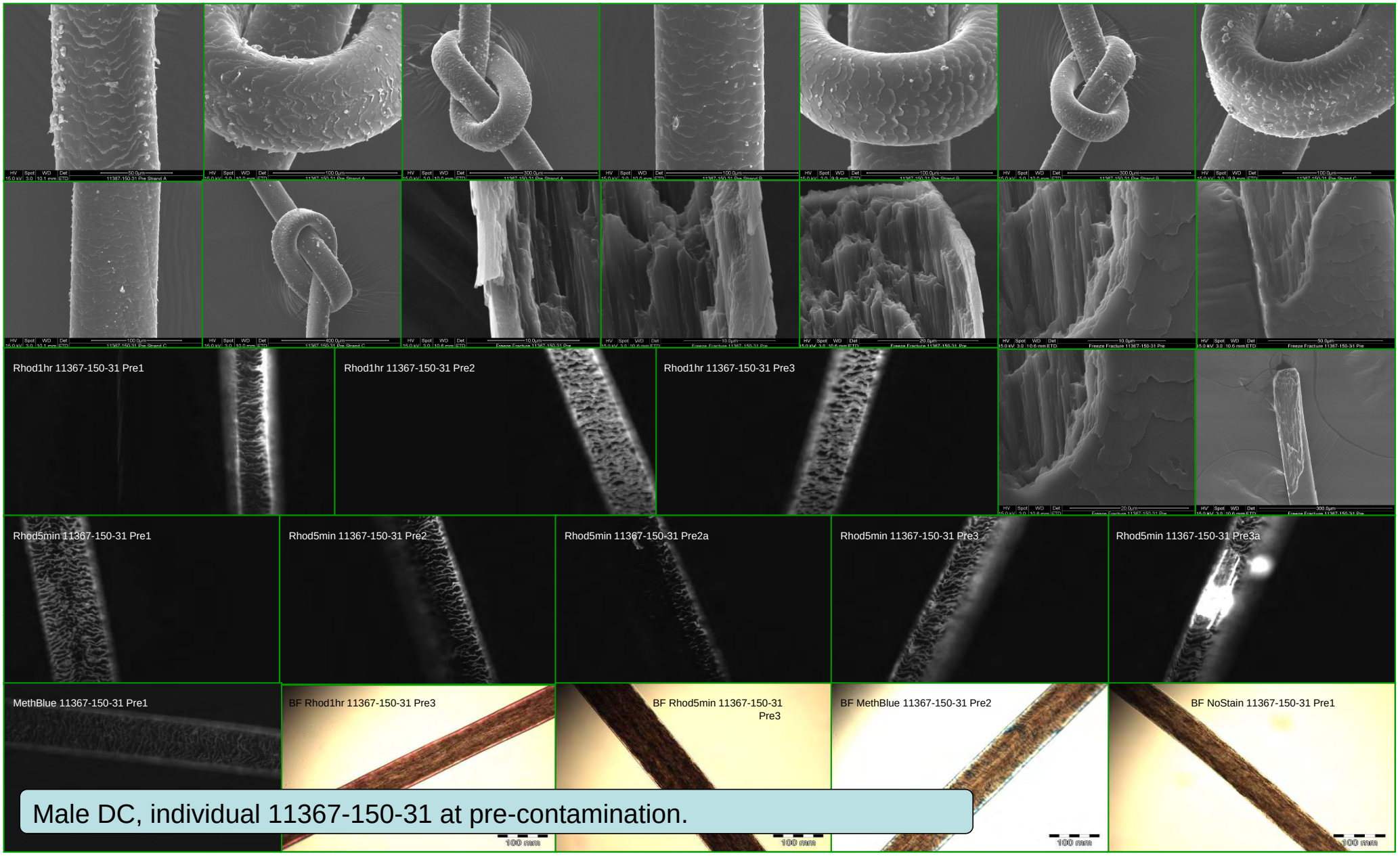

Rhod1hr 11367-150-27 Pre1
Rhod1hr 11367-150-27 Pre1a
Rhod1hr 11367-150-27 Pre2
Rhod1hr 11367-150-27 Pre2a
Rhod1hr 11367-150-27 Pre3
Rhod1hr 11367-150-27 Pre13a
Rhod5min 11367-150-27 Pre1
Rhod5min 11367-150-27 Pre1a
Rhod5min 11367-150-27 Pre2
Rhod5min 11367-150-27 Pre2a
Rhod5min 11367-150-27 Pre3
MethBlue 11367-150-27 Pre2
BF Rhod1hr 11367-150-27 Pre3
BF Rhod5min 11367-150-27 Pre1
BF MethBlue 11367-150-27 Pre2
BF NoStain 11367-150-27 Pre1
100 mm
100 mm
100 µm
100 mm
Male DC, individual 11367-150-27 at pre-contamination.

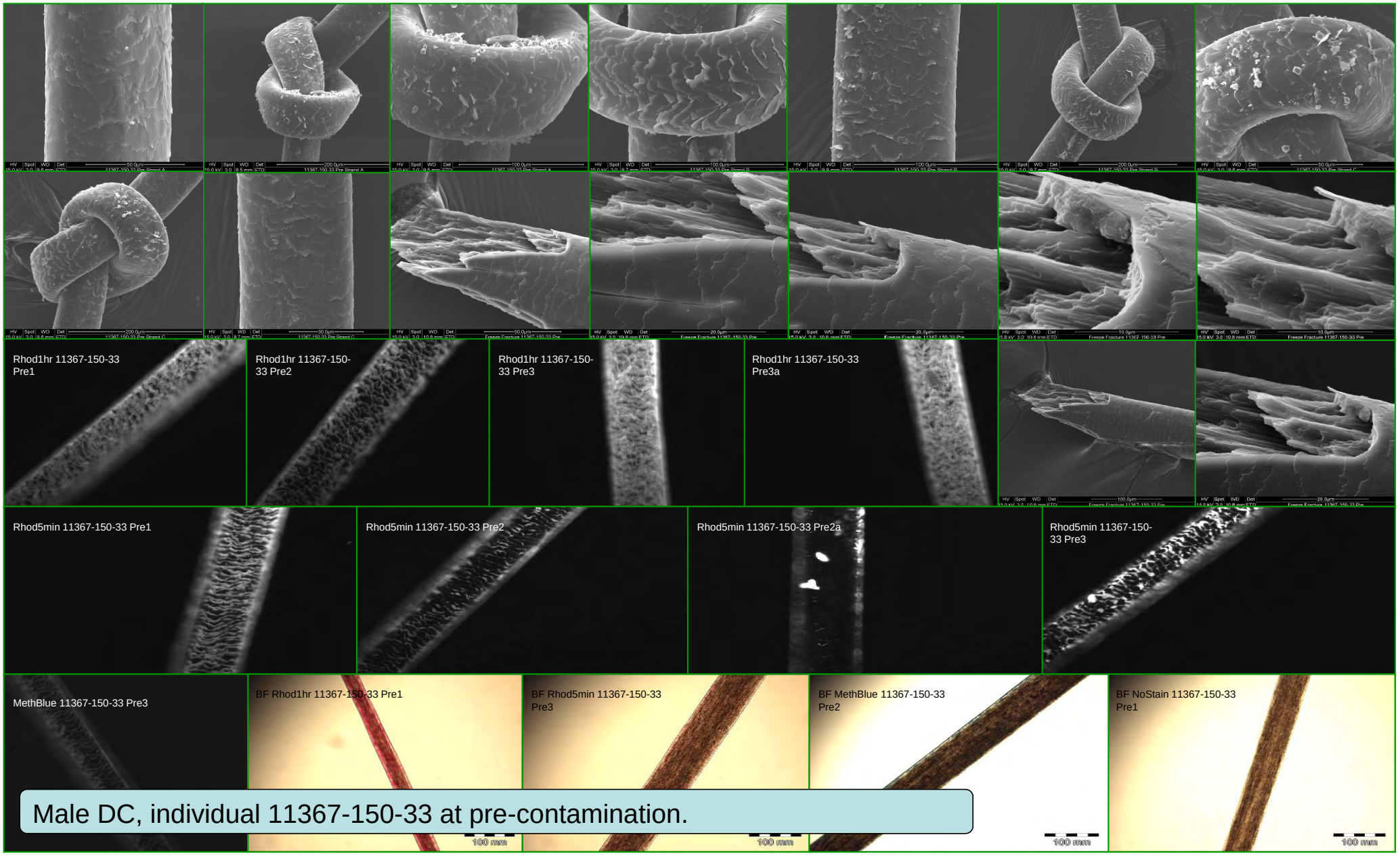

Rhod1hr 11367-150-28 Pre1
Rhod1hr 11367-150-28 Pre2
Rhod1hr 11367-150-28 Pre3
Rhod1hr 11367-150-28 Pre3a
Rhod5min 11367-150-28 Pre1
Rhod5min 11367-150-28 Pre2
Rhod5min 11367-150-28 Pre2a
Rhod5min 11367-150-28 Pre3
Rhod5min 11367-150-28 Pre3a
MethBlue 11367-150-28 Pre1
BF Rhod1hr 11367-150-28 Pre3
BF Rhod5min 11367-150-28 Pre1
BF MethBlue 11367-150-28 Pre3
BF NoStain 11367-150-28 Pre1
100 mm
100 mm
100 mm
100 mm
Female DC, individual 11367-150-28 at pre-contamination.

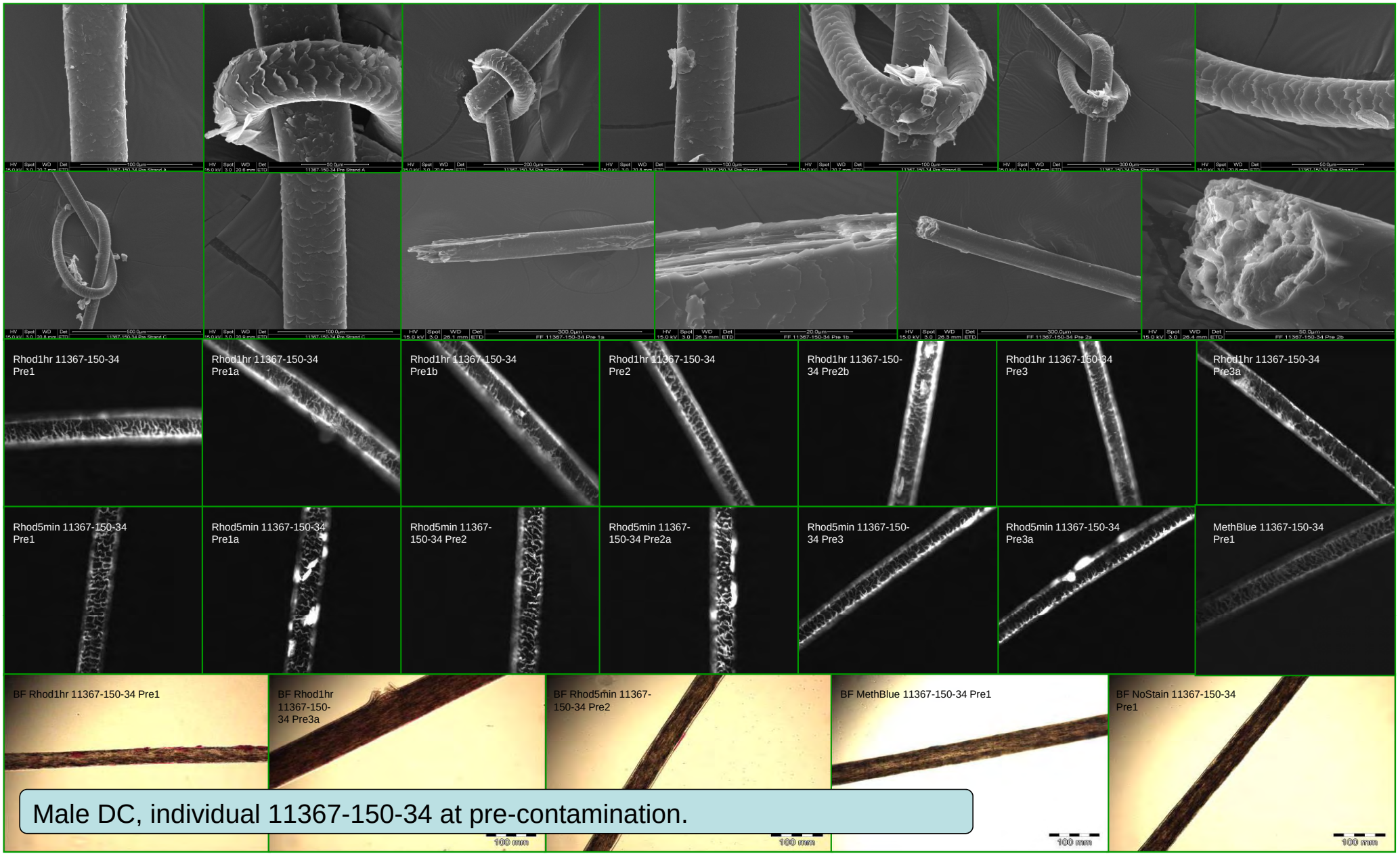

Rhod1hr 11367-150-29 Pre1
Rhod1hr 11367-150-29 Pre1a
Rhod1hr 11367-150-29 Pre2
Rhod1hr 11367-150-29 Pre2a
Rhod1hr 11367-150-29 Pre3
Rhod1hr 11367-150-29 Pre3a
Rhod5min 11367-150-29 Pre1
Rhod5min 11367-150-29 Pre1a
Rhod5min 11367-150-29 Pre2
Rhod5min 11367-150-29 Pre12a
Rhod5min 11367-150-29 Pre3
Rhod5min 11367-150-29 Pre3a
MethBlue 11367-150-29 Pre1
BF Rhod1hr 11367-150-29 Pre2
BF Rhod5min 11367-150-29 Pre2
BF MethBlue 11367-150-29 Pre3
BF NoStain 11367-150-29 Pre1
100 mm
100 mm
100 mm
100 mm
Male DC, individual 11367-150-29 at pre-contamination.

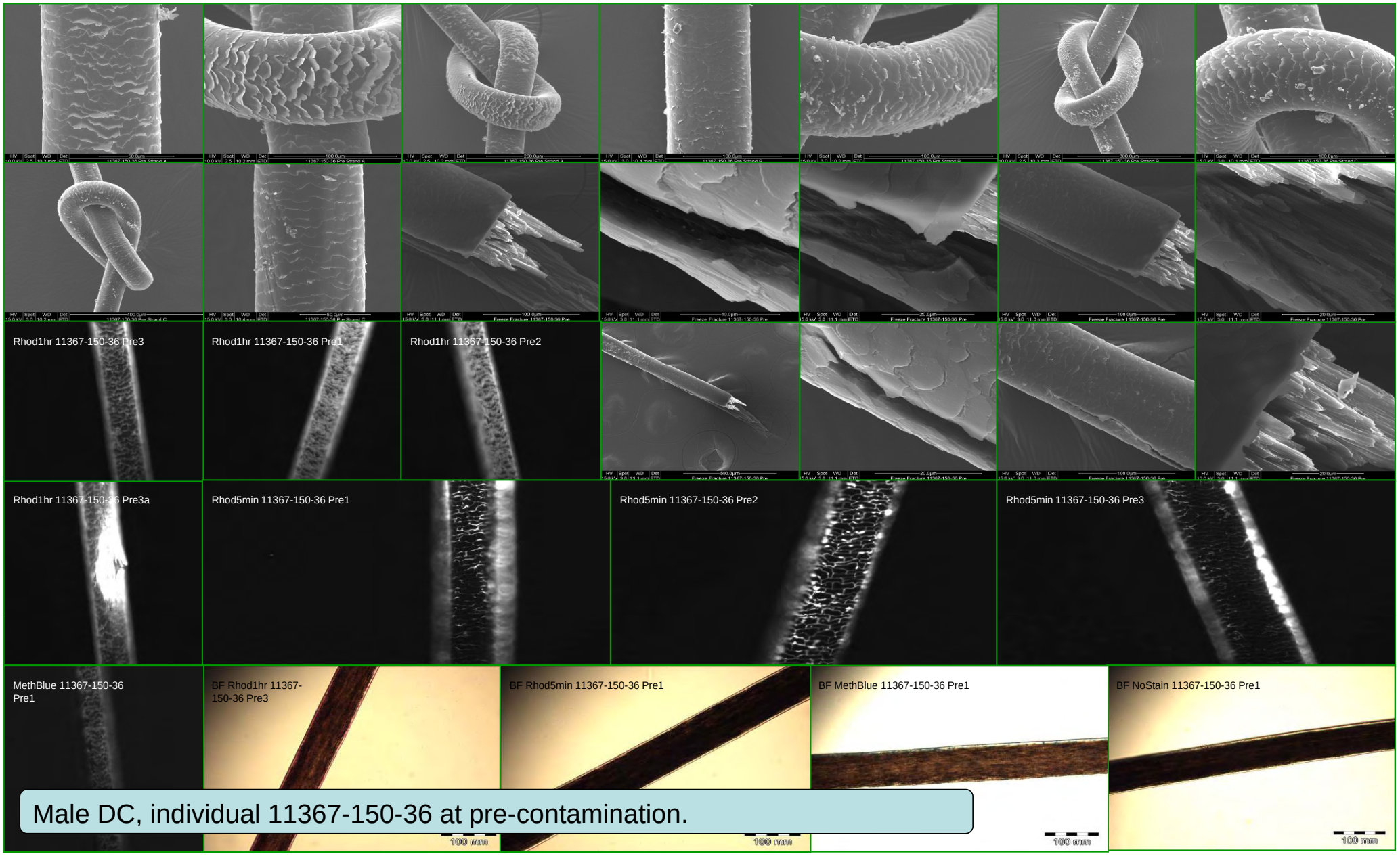


Female DC, individual 11367-150-30 at pre-contamination.

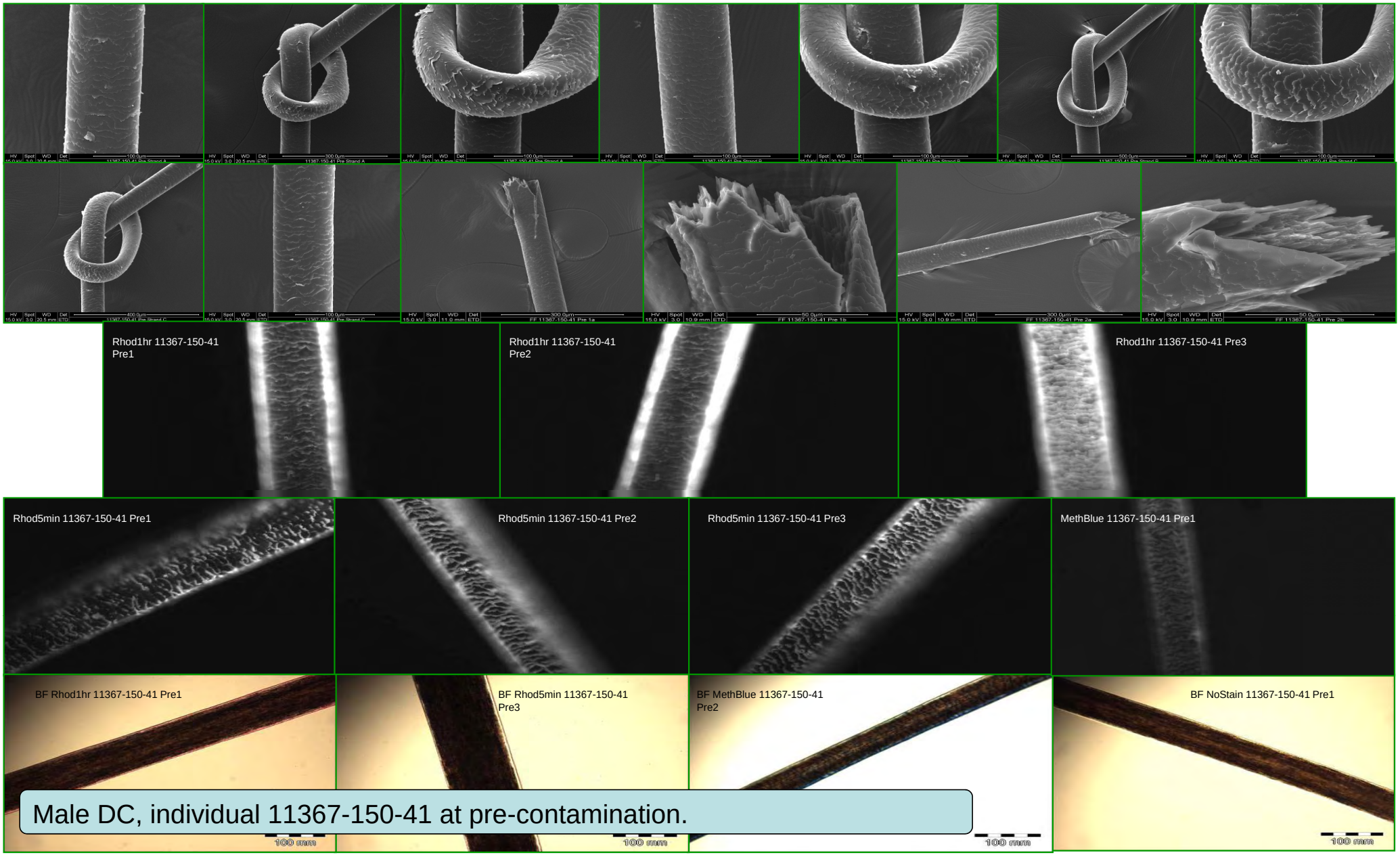

Rhod1hr 11367-150-31 Pre1
Rhod1hr 11367-150-31 Pre2
Rhod1hr 11367-150-31 Pre3
Rhod5min 11367-150-31 Pre1
Rhod5min 11367-150-31 Pre2
Rhod5min 11367-150-31 Pre2a
Rhod5min 11367-150-31 Pre3
Rhod5min 11367-150-31 Pre3a
MethBlue 11367-150-31 Pre1
BF Rhod1hr 11367-150-31 Pre3
BF Rhod5min 11367-150-31 Pre3
BF MethBlue 11367-150-31 Pre2
BF NoStain 11367-150-31 Pre1
100 mm
Male DC, individual 11367-150-31 at pre-contamination.

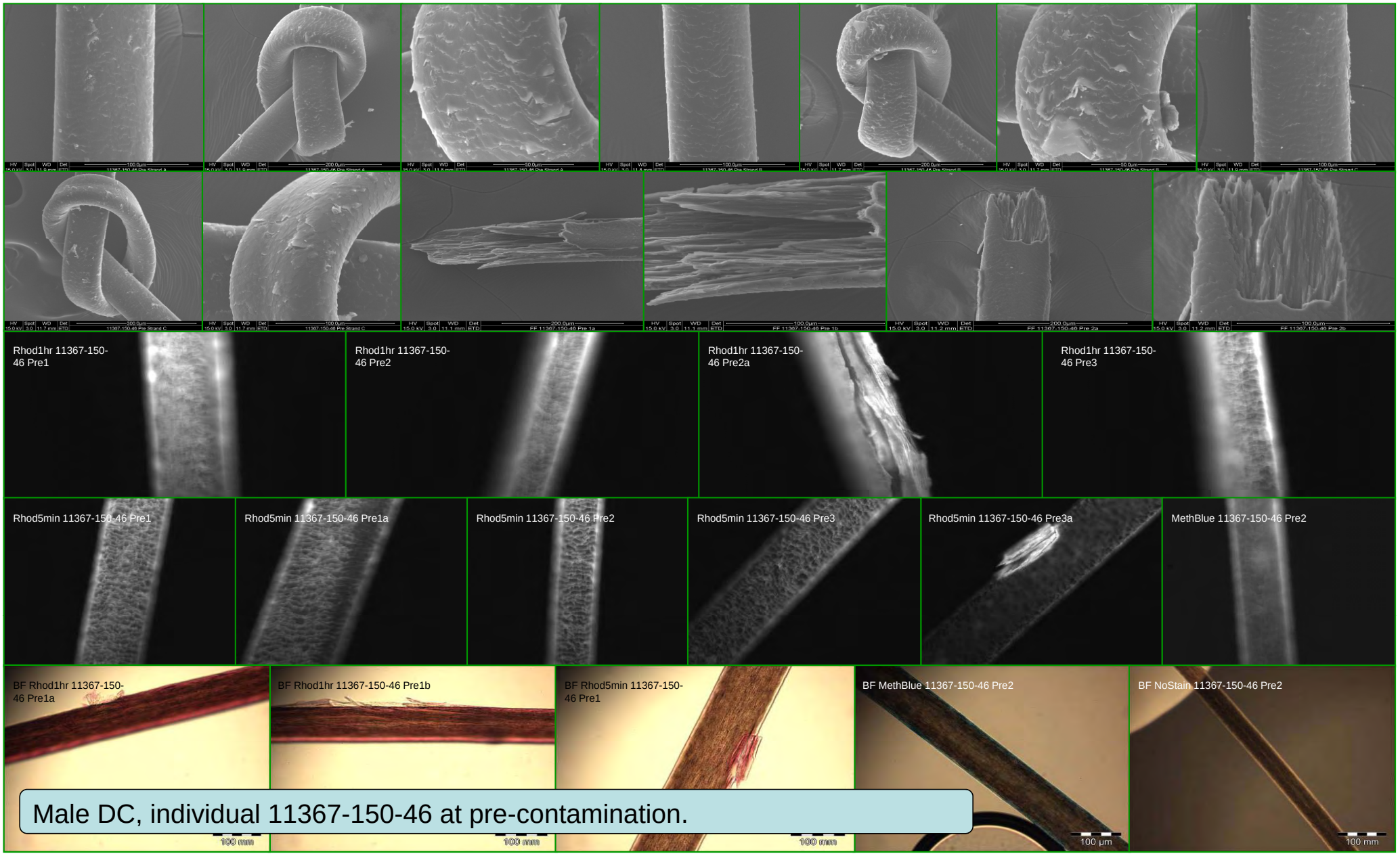

Rhod1hr 11367-150-33 Pre1
Rhod1hr 11367-150-33 Pre2
Rhod1hr 11367-150-33 Pre3
Rhod1hr 11367-150-33 Pre3a
Rhod5min 11367-150-33 Pre1
Rhod5min 11367-150-33 Pre2
Rhod5min 11367-150-33 Pre2a
Rhod5min 11367-150-33 Pre3
MethBlue 11367-150-33 Pre3
BF Rhod1hr 11367-150-33 Pre1
BF Rhod5min 11367-150-33 Pre3
BF MethBlue 11367-150-33 Pre2
BF NoStain 11367-150-33 Pre1
100 mm
Male DC, individual 11367-150-33 at pre-contamination.


Rhod1hr 11367-150-34 Pre1
Rhod1hr 11367-150-34 Pre1a
Rhod1hr 11367-150-34 Pre1b
Rhod1hr 11367-150-34 Pre2
Rhod1hr 11367-150-34 Pre2b
Rhod1hr 11367-150-34 Pre3
Rhod1hr 11367-150-34 Pre3a
Rhod5min 11367-150-34 Pre1
Rhod5min 11367-150-34 Pre1a
Rhod5min 11367-150-34 Pre2
Rhod5min 11367-150-34 Pre2a
Rhod5min 11367-150-34 Pre3
Rhod5min 11367-150-34 Pre3a
MethBlue 11367-150-34 Pre1
BF Rhod1hr 11367-150-34 Pre1
BF Rhod1hr 11367-150-34 Pre3a
BF Rhod5min 11367-150-34 Pre2
BF MethBlue 11367-150-34 Pre1
BF NoStain 11367-150-34 Pre1
100 mm
100 mm
100 mm
100 mm
Male DC, individual 11367-150-34 at pre-contamination.



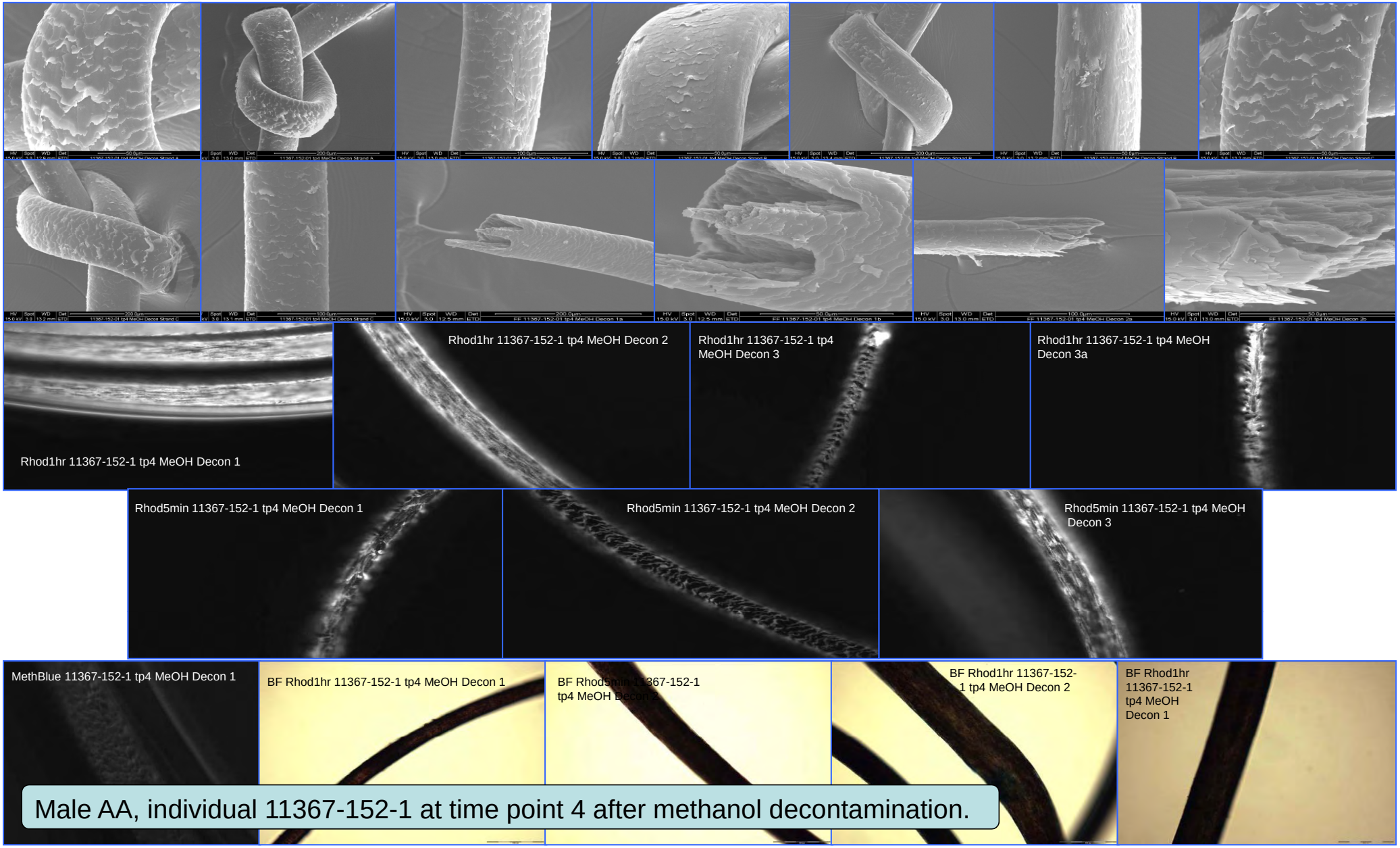
Male DC, individual 11367-150-36 at pre-contamination.

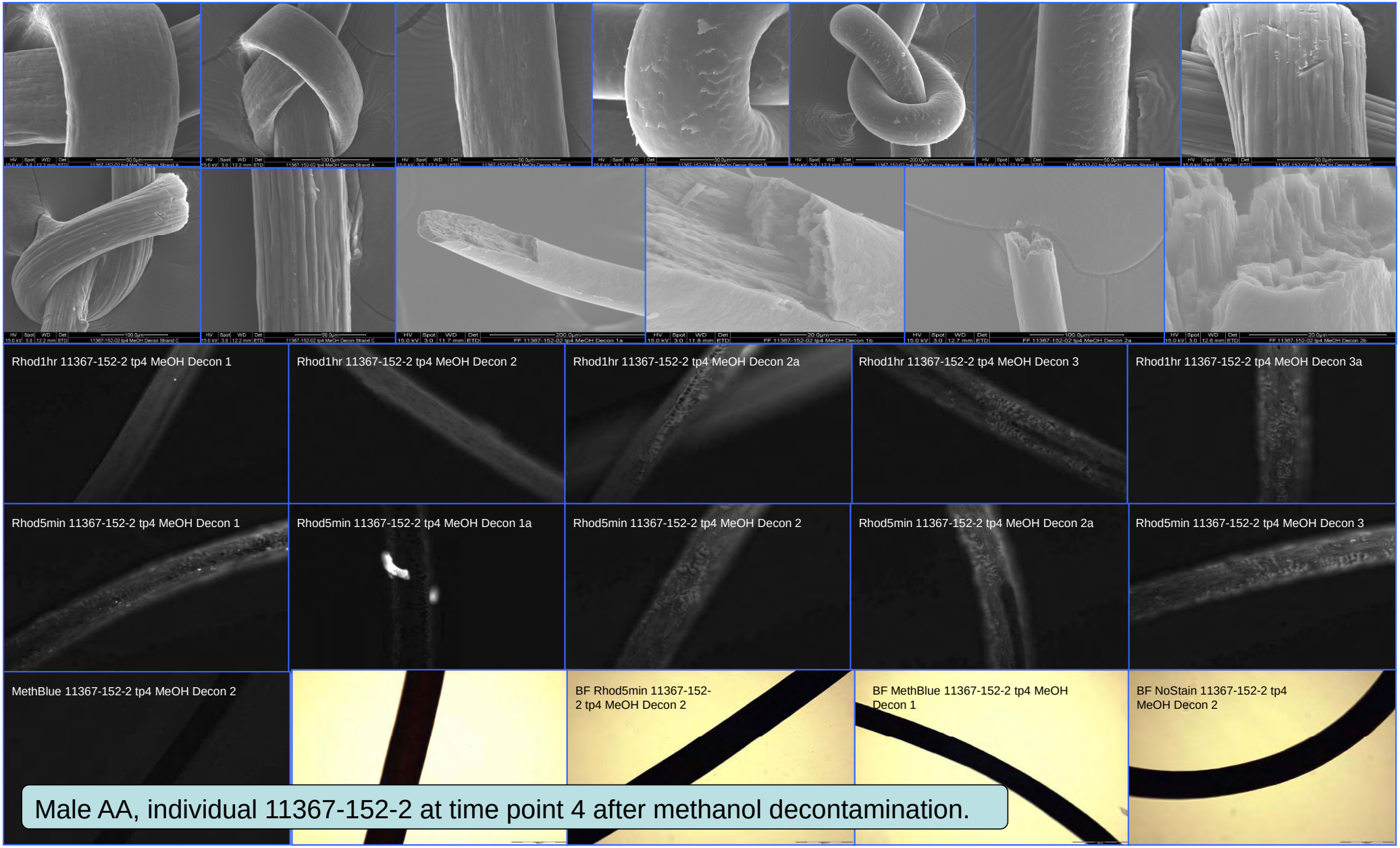

Rhod1hr 11367-150-41 Pre1
Rhod1hr 11367-150-41 Pre2
Rhod1hr 11367-150-41 Pre3
Rhod5min 11367-150-41 Pre1
Rhod5min 11367-150-41 Pre2
Rhod5min 11367-150-41 Pre3
MethBlue 11367-150-41 Pre1
BF Rhod1hr 11367-150-41 Pre1
BF Rhod5min 11367-150-41 Pre3
BF MethBlue 11367-150-41 Pre2
BF NoStain 11367-150-41 Pre1
100 mm
Male DC, individual 11367-150-41 at pre-contamination.

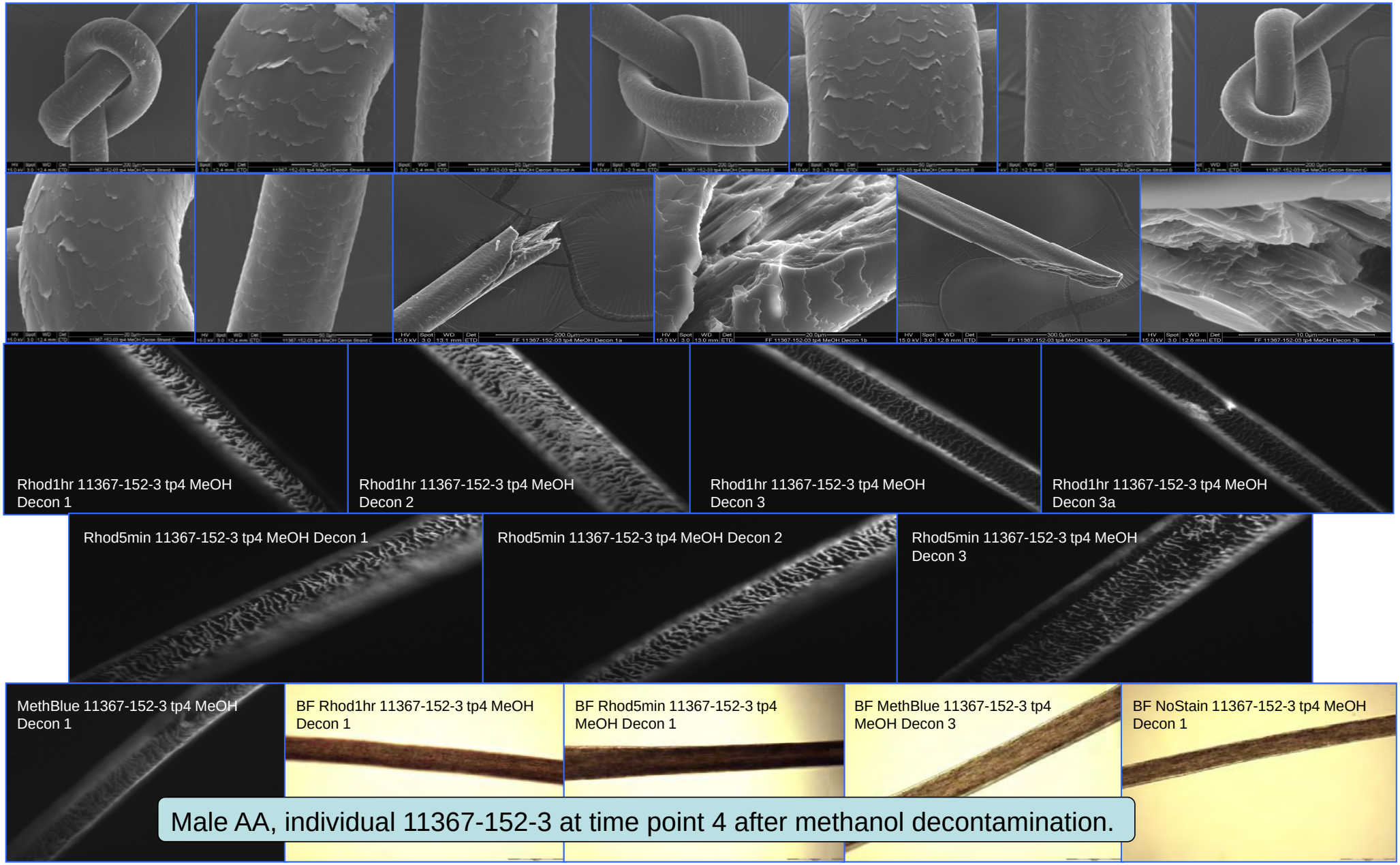

Rhod1hr 11367-150-46 Pre1
Rhod1hr 11367-150-46 Pre2
Rhod1hr 11367-150-46 Pre2a
Rhod1hr 11367-150-46 Pre3
Rhod5min 11367-150-46 Pre1
Rhod5min 11367-150-46 Pre1a
Rhod5min 11367-150-46 Pre2
Rhod5min 11367-150-46 Pre3
Rhod5min 11367-150-46 Pre3a
MethBlue 11367-150-46 Pre2
BF Rhod1hr 11367-150-46 Pre1a
BF Rhod1hr 11367-150-46 Pre1b
BF Rhod5min 11367-150-46 Pre1
BF MethBlue 11367-150-46 Pre2
BF NoStain 11367-150-46 Pre2
100 mm
100 mm
100 mm
100 µm
100 mm
Male DC, individual 11367-150-46 at pre-contamination.

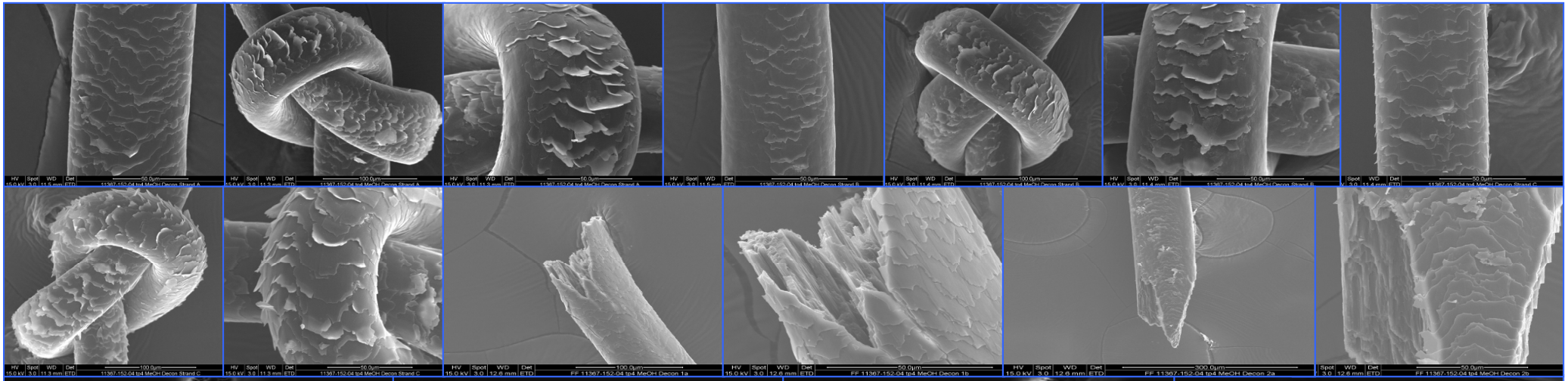
Appendix C, Part 2

Individuals at Time Point 4 After Methanol Decontamination


Rhod1hr 11367-152-1 tp4 MeOH Decon 1
Rhod1hr 11367-152-1 tp4 MeOH Decon 2
Rhod1hr 11367-152-1 tp4 MeOH Decon 3
Rhod1hr 11367-152-1 tp4 MeOH Decon 3a
Rhod5min 11367-152-1 tp4 MeOH Decon 1
Rhod5min 11367-152-1 tp4 MeOH Decon 2
Rhod5min 11367-152-1 tp4 MeOH Decon 3
MethBlue 11367-152-1 tp4 MeOH Decon 1
BF Rhod1hr 11367-152-1 tp4 MeOH Decon 1
BF Rhod1hr 11367-152-1 tp4 MeOH Decon 2
BF Rhod1hr 11367-152-1 tp4 MeOH Decon 1
Male AA, individual 11367-152-1 at time point 4 after methanol decontamination.


Rhod1hr 11367-152-2 tp4 MeOH Decon 1
Rhod1hr 11367-152-2 tp4 MeOH Decon 2
Rhod1hr 11367-152-2 tp4 MeOH Decon 2a
Rhod1hr 11367-152-2 tp4 MeOH Decon 3
Rhod1hr 11367-152-2 tp4 MeOH Decon 3a
Rhod5min 11367-152-2 tp4 MeOH Decon 1
Rhod5min 11367-152-2 tp4 MeOH Decon 1a
Rhod5min 11367-152-2 tp4 MeOH Decon 2
Rhod5min 11367-152-2 tp4 MeOH Decon 2a
Rhod5min 11367-152-2 tp4 MeOH Decon 3
MethBlue 11367-152-2 tp4 MeOH Decon 2
BF Rhod5min 11367-152-2 tp4 MeOH Decon 2
BF MethBlue 11367-152-2 tp4 MeOH Decon 1
BF NoStain 11367-152-2 tp4 MeOH Decon 2
Male AA, individual 11367-152-2 at time point 4 after methanol decontamination.


Rhod1hr 11367-152-3 tp4 MeOH Decon 1
Rhod1hr 11367-152-3 tp4 MeOH Decon 2
Rhod1hr 11367-152-3 tp4 MeOH Decon 3
Rhod1hr 11367-152-3 tp4 MeOH Decon 3a
Rhod5min 11367-152-3 tp4 MeOH Decon 1
Rhod5min 11367-152-3 tp4 MeOH Decon 2
Rhod5min 11367-152-3 tp4 MeOH Decon 3
MethBlue 11367-152-3 tp4 MeOH Decon 1
BF Rhod1hr 11367-152-3 tp4 MeOH Decon 1
BF Rhod5min 11367-152-3 tp4 MeOH Decon 1
BF MethBlue 11367-152-3 tp4 MeOH Decon 3
BF NoStain 11367-152-3 tp4 MeOH Decon 1
Male AA, individual 11367-152-3 at time point 4 after methanol decontamination.



Rhod1hr 11367-152-4 tp4 MeOH Decon 1

Rhod1hr 11367-152-4 tp4 MeOH Decon 2

Rhod1hr 11367-152-4 tp4 MeOH Decon 2a

Rhod1hr 11367-152-4 tp4 MeOH Decon 3

Rhod5min 11367-152-4 tp4 MeOH Decon 1

Rhod5min 11367-152-4 tp4 MeOH Decon 1a

Rhod5min 11367-152-4 tp4 MeOH Decon 2

Rhod5min 11367-152-4 tp4 MeOH Decon 2a

Rhod5min 11367-152-4 tp4 MeOH Decon 3

MethBlue 11367-152-4 tp4 MeOH Decon 1

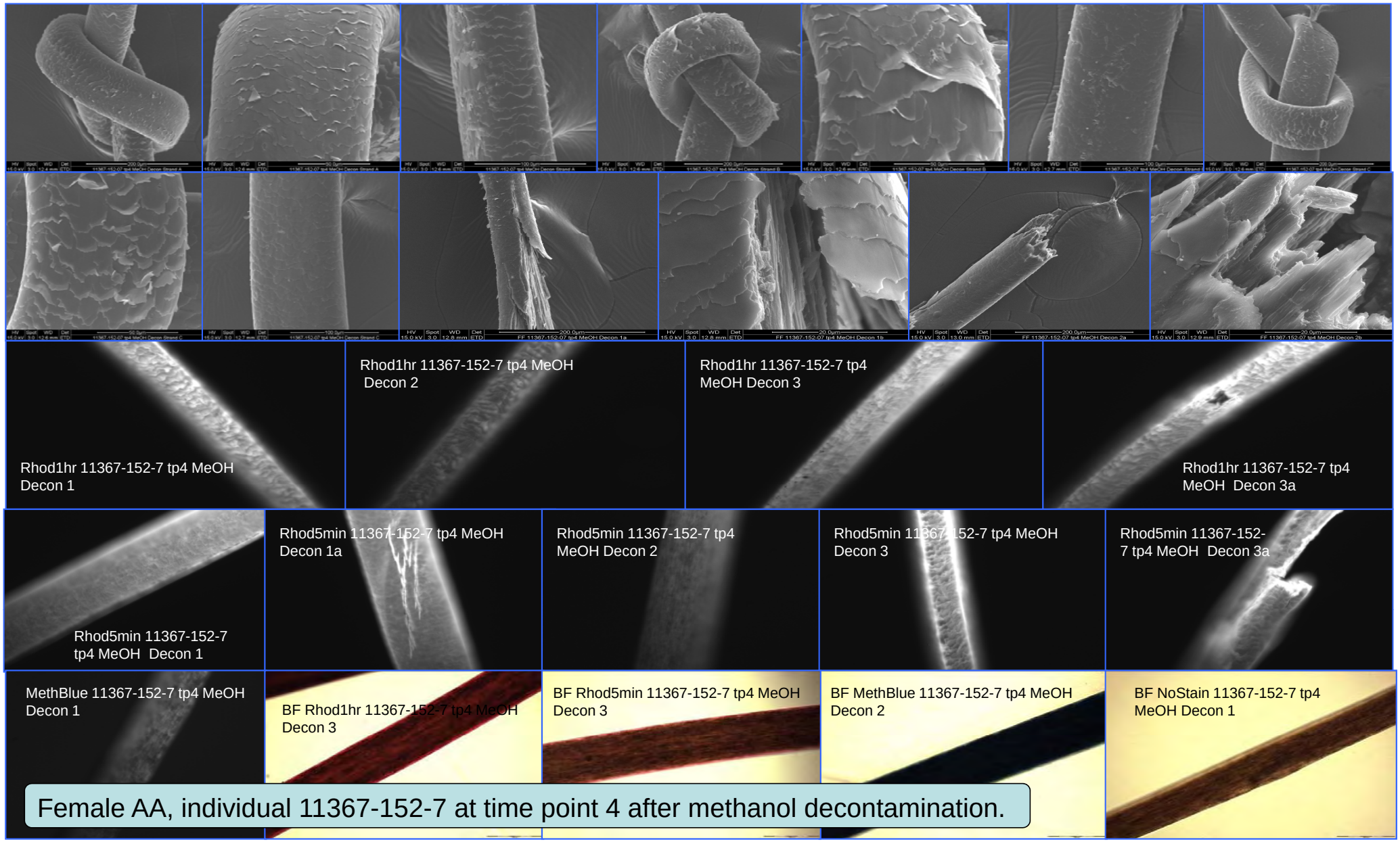
BF Rhod5min 11367-152 4 tp4 MeOH Decon 2

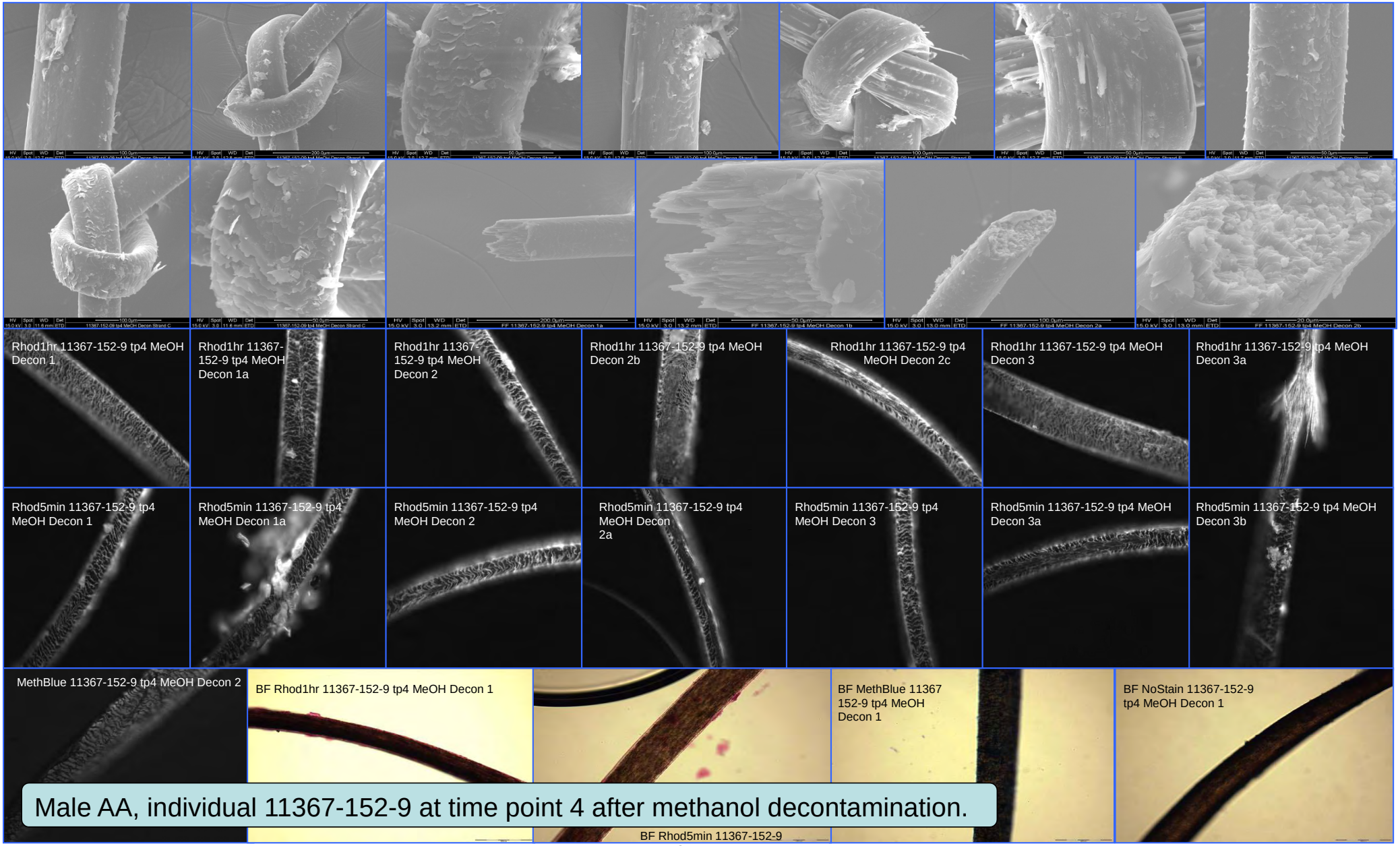
BF Rhod5min 11367-152-4 tp4 MeOH Decon 1

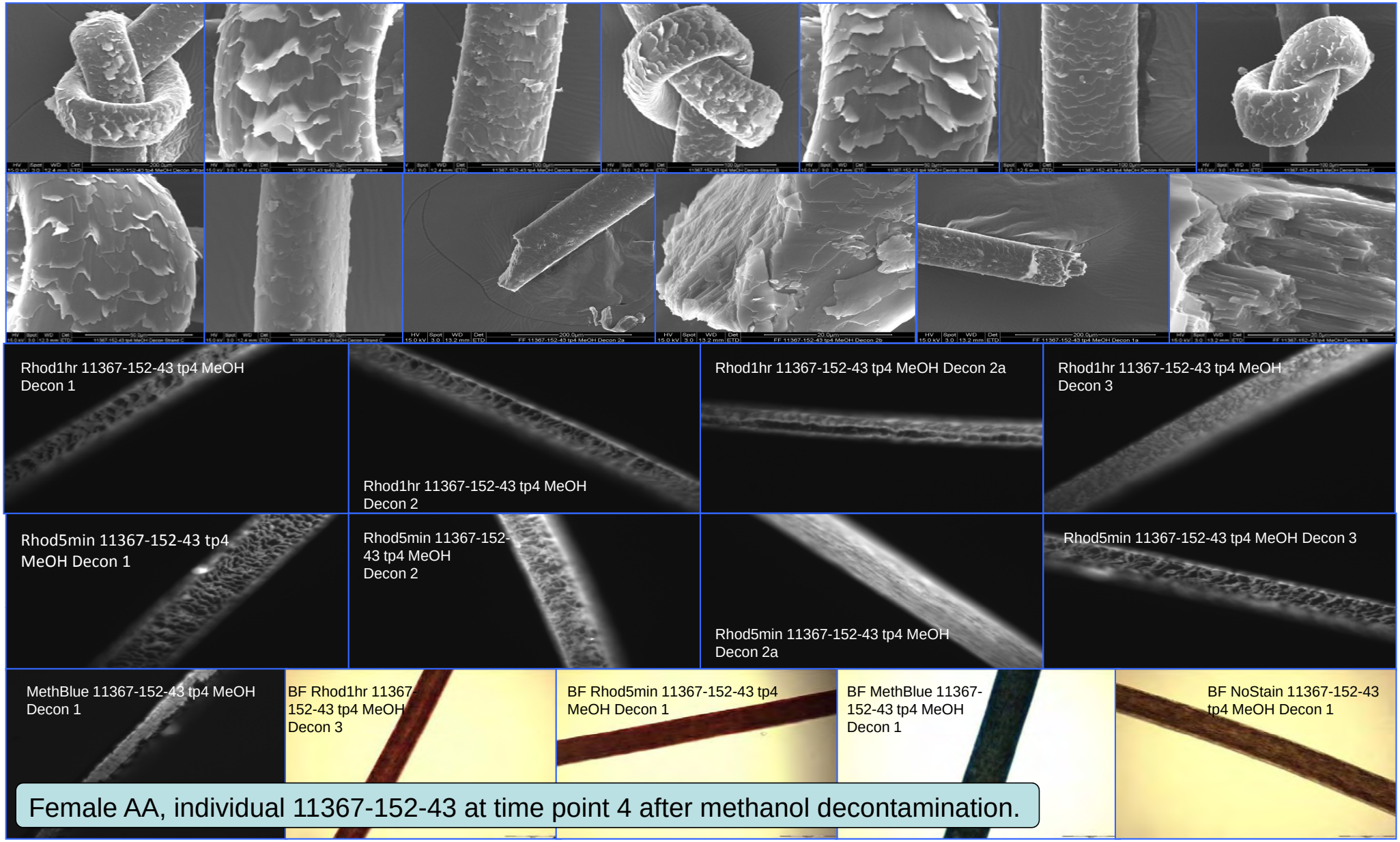
BF MethBlue 11367-152-4 tp4 MeOH Decon 2

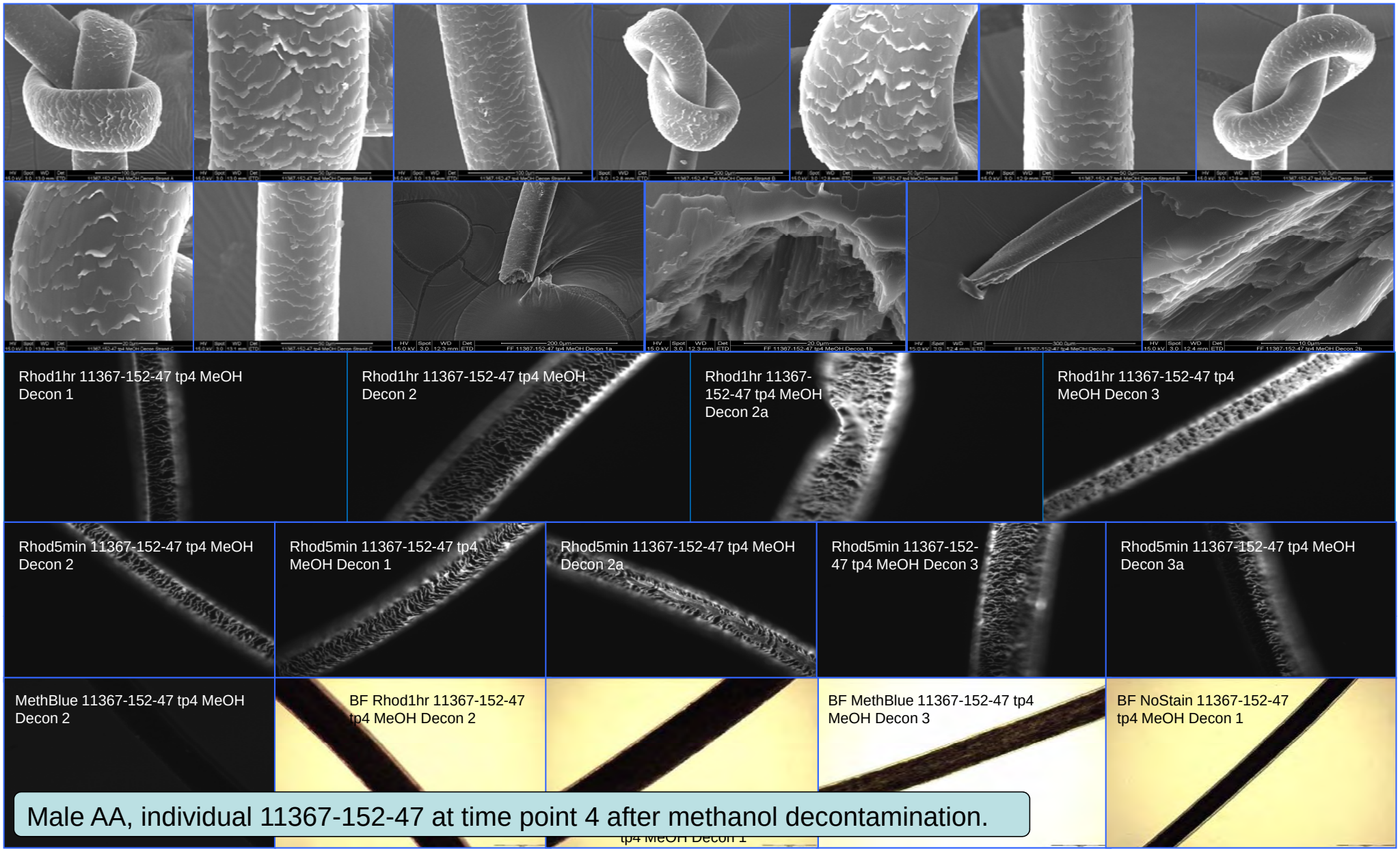
BF NoStain 11367-152-4 tp4 MeOH Decon 1

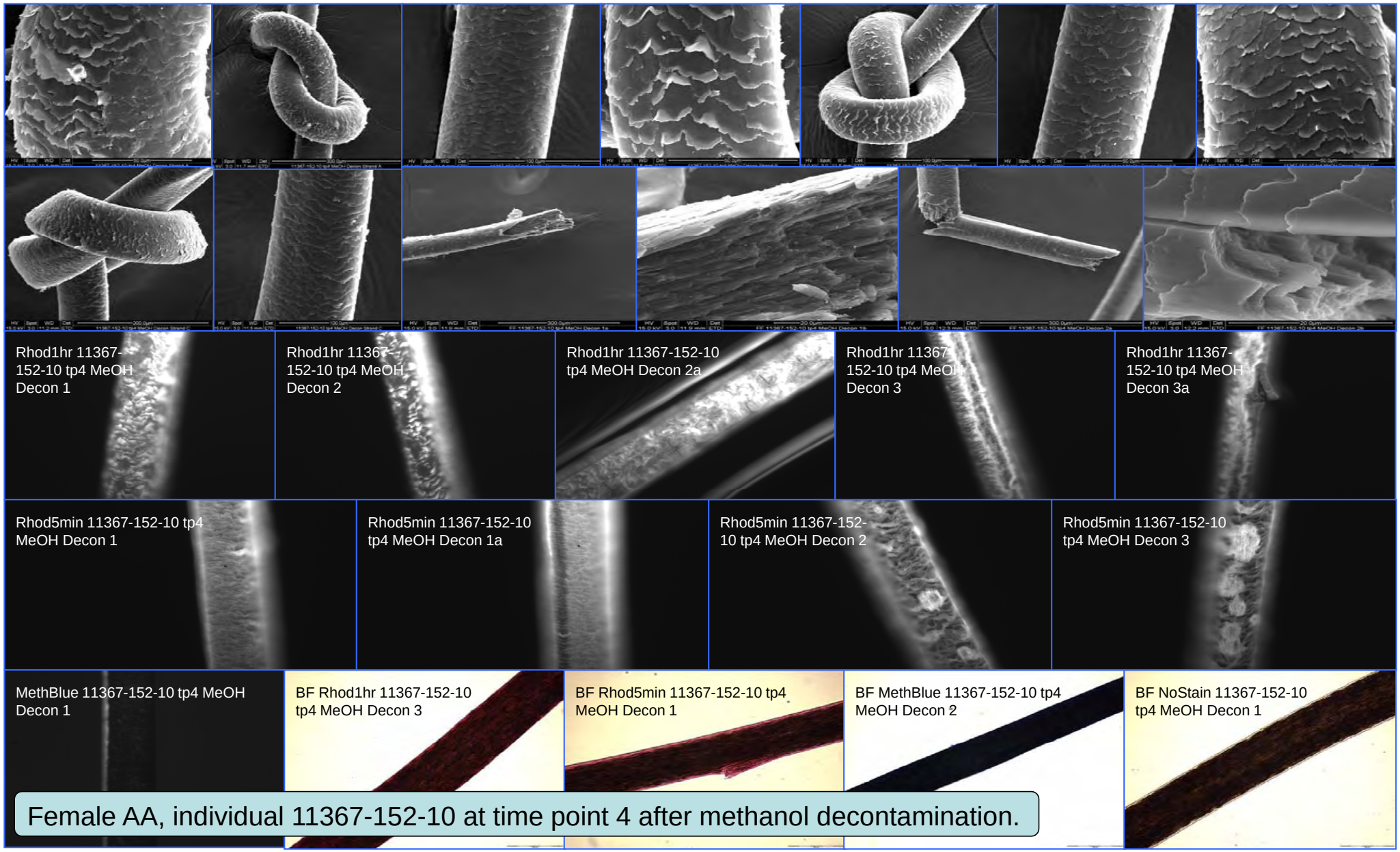
Male AA, individual 11367-152-4 at time point 4 after methanol decontamination.

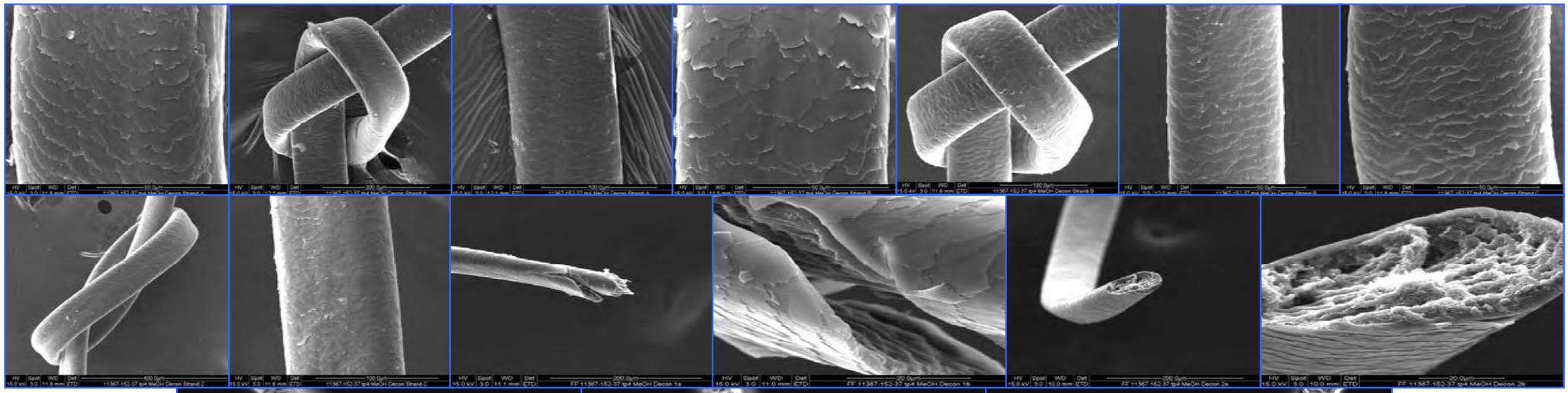

Rhod1hr 11367-152-7 tp4 MeOH Decon 1
Rhod1hr 11367-152-7 tp4 MeOH Decon 2
Rhod1hr 11367-152-7 tp4 MeOH Decon 3
Rhod1hr 11367-152-7 tp4 MeOH Decon 3a
Rhod5min 11367-152-7 tp4 MeOH Decon 1
Rhod5min 11367-152-7 tp4 MeOH Decon 1a
Rhod5min 11367-152-7 tp4 MeOH Decon 2
Rhod5min 11367-152-7 tp4 MeOH Decon 3
Rhod5min 11367-152-7 tp4 MeOH Decon 3a
MethBlue 11367-152-7 tp4 MeOH Decon 1
BF Rhod1hr 11367-152-7 tp4 MeOH Decon 3
BF Rhod5min 11367-152-7 tp4 MeOH Decon 3
BF MethBlue 11367-152-7 tp4 MeOH Decon 2
BF NoStain 11367-152-7 tp4 MeOH Decon 1
Female AA, individual 11367-152-7 at time point 4 after methanol decontamination.

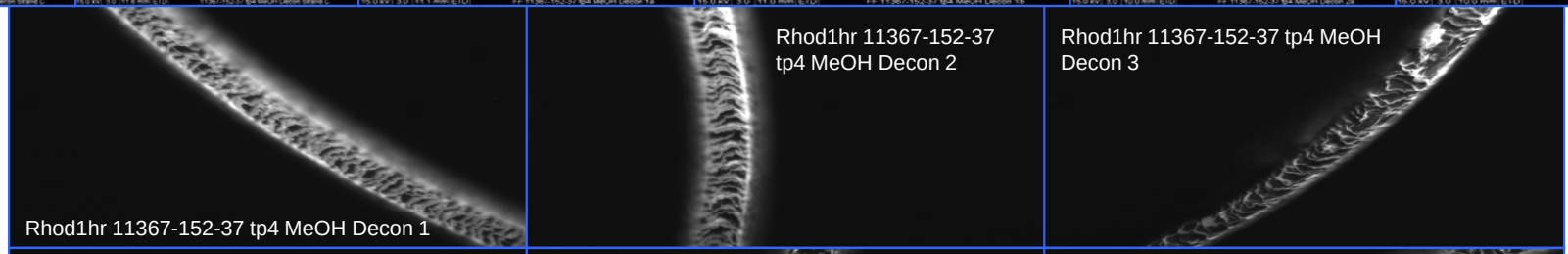

Rhod1hr 11367-152-9 tp4 MeOH Decon 1
Rhod1hr 11367-152-9 tp4 MeOH Decon 1a
Rhod1hr 11367-152-9 tp4 MeOH Decon 2
Rhod1hr 11367-152-9 tp4 MeOH Decon 2b
Rhod1hr 11367-152-9 tp4 MeOH Decon 2c
Rhod1hr 11367-152-9 tp4 MeOH Decon 3
Rhod1hr 11367-152-9 tp4 MeOH Decon 3a
Rhod5min 11367-152-9 tp4 MeOH Decon 1
Rhod5min 11367-152-9 tp4 MeOH Decon 1a
Rhod5min 11367-152-9 tp4 MeOH Decon 2
Rhod5min 11367-152-9 tp4 MeOH Decon 2a
Rhod5min 11367-152-9 tp4 MeOH Decon 3
Rhod5min 11367-152-9 tp4 MeOH Decon 3a
Rhod5min 11367-152-9 tp4 MeOH Decon 3b
MethBlue 11367-152-9 tp4 MeOH Decon 2
BF Rhod1hr 11367-152-9 tp4 MeOH Decon 1
BF MethBlue 11367 152-9 tp4 MeOH Decon 1
BF NoStain 11367-152-9 tp4 MeOH Decon 1
Male AA, individual 11367-152-9 at time point 4 after methanol decontamination.
BF Rhod5min 11367-152-9 tp4 MeOH Decon 1

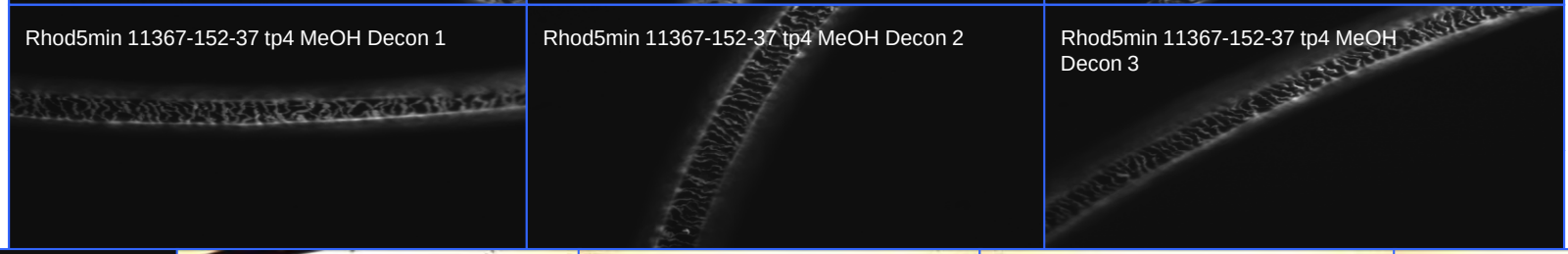

Rhod1hr 11367-152-43 tp4 MeOH Decon 1
Rhod1hr 11367-152-43 tp4 MeOH Decon 2
Rhod1hr 11367-152-43 tp4 MeOH Decon 2a
Rhod1hr 11367-152-43 tp4 MeOH Decon 3
Rhod5min 11367-152-43 tp4 MeOH Decon 1
Rhod5min 11367-152-43 tp4 MeOH Decon 2
Rhod5min 11367-152-43 tp4 MeOH Decon 2a
Rhod5min 11367-152-43 tp4 MeOH Decon 3
MethBlue 11367-152-43 tp4 MeOH Decon 1
BF Rhod1hr 11367-152-43 tp4 MeOH Decon 3
BF Rhod5min 11367-152-43 tp4 MeOH Decon 1
BF MethBlue 11367-152-43 tp4 MeOH Decon 1
BF NoStain 11367-152-43 tp4 MeOH Decon 1
Female AA, individual 11367-152-43 at time point 4 after methanol decontamination.

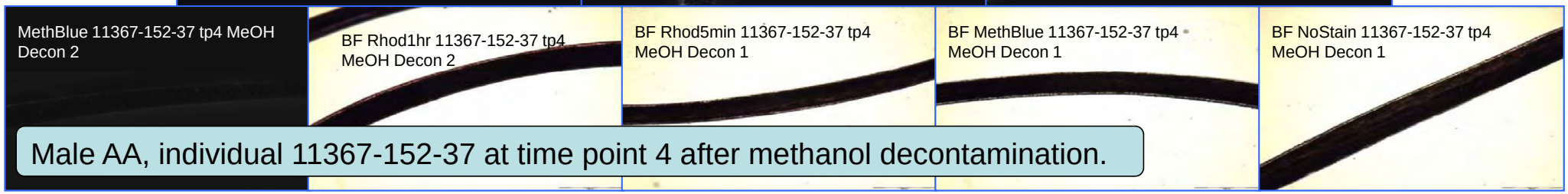

Rhod1hr 11367-152-47 tp4 MeOH Decon 1
Rhod1hr 11367-152-47 tp4 MeOH Decon 2
Rhod1hr 11367-152-47 tp4 MeOH Decon 2a
Rhod1hr 11367-152-47 tp4 MeOH Decon 3
Rhod5min 11367-152-47 tp4 MeOH Decon 2
Rhod5min 11367-152-47 tp4 MeOH Decon 1
Rhod5min 11367-152-47 tp4 MeOH Decon 2a
Rhod5min 11367-152-47 tp4 MeOH Decon 3
Rhod5min 11367-152-47 tp4 MeOH Decon 3a
MethBlue 11367-152-47 tp4 MeOH Decon 2
BF Rhod1hr 11367-152-47 tp4 MeOH Decon 2
BF MethBlue 11367-152-47 tp4 MeOH Decon 3
BF NoStain 11367-152-47 tp4 MeOH Decon 1
Male AA, individual 11367-152-47 at time point 4 after methanol decontamination.


Rhod1hr 11367-152-10 tp4 MeOH Decon 1
Rhod1hr 11367-152-10 tp4 MeOH Decon 2
Rhod1hr 11367-152-10 tp4 MeOH Decon 2a
Rhod1hr 11367-152-10 tp4 MeOH Decon 3
Rhod1hr 11367-152-10 tp4 MeOH Decon 3a
Rhod5min 11367-152-10 tp4 MeOH Decon 1
Rhod5min 11367-152-10 tp4 MeOH Decon 1a
Rhod5min 11367-152-10 tp4 MeOH Decon 2
Rhod5min 11367-152-10 tp4 MeOH Decon 3
MethBlue 11367-152-10 tp4 MeOH Decon 1
BF Rhod1hr 11367-152-10 tp4 MeOH Decon 3
BF Rhod5min 11367-152-10 tp4 MeOH Decon 1
BF MethBlue 11367-152-10 tp4 MeOH Decon 2
BF NoStain 11367-152-10 tp4 MeOH Decon 1
Female AA, individual 11367-152-10 at time point 4 after methanol decontamination.

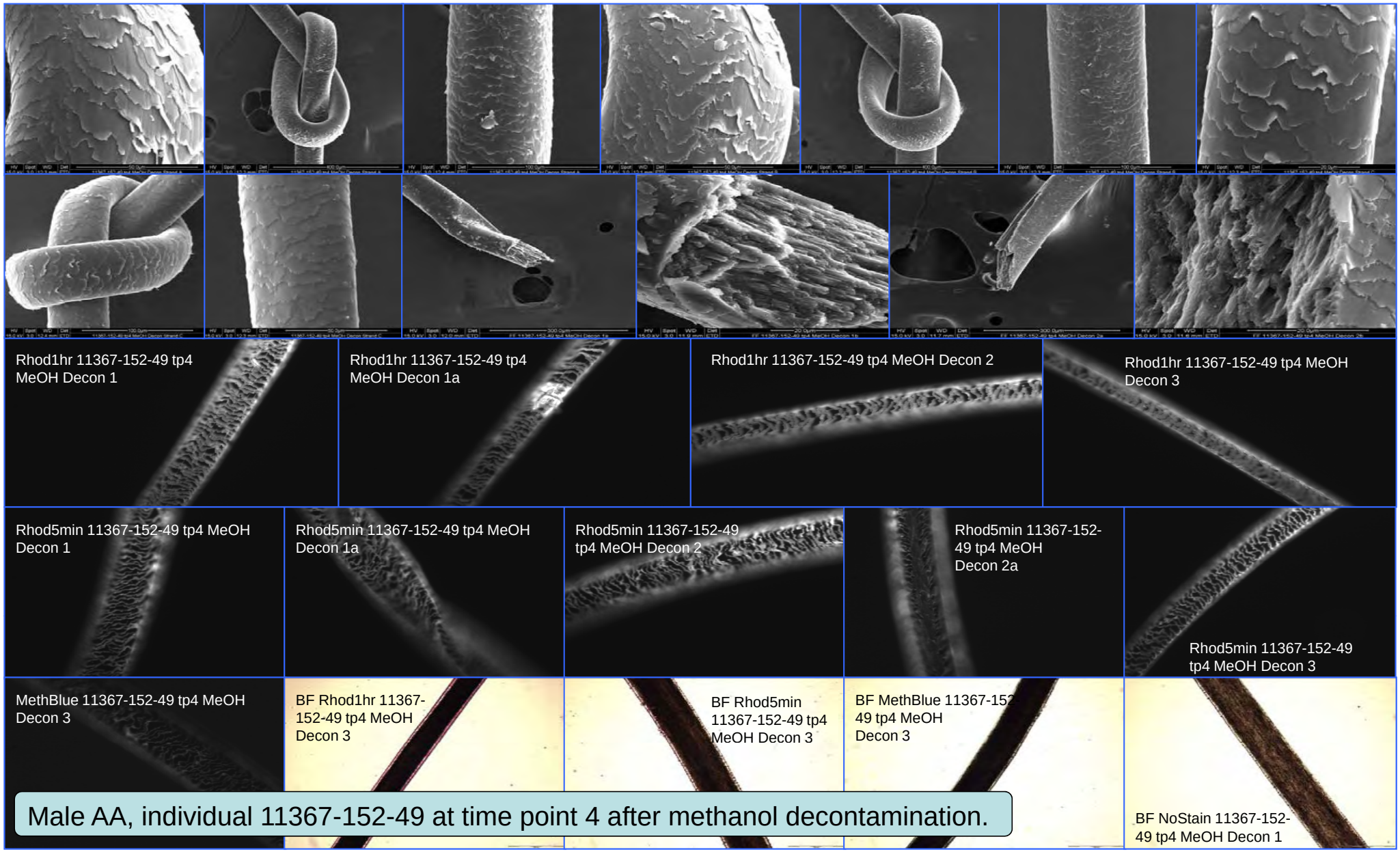


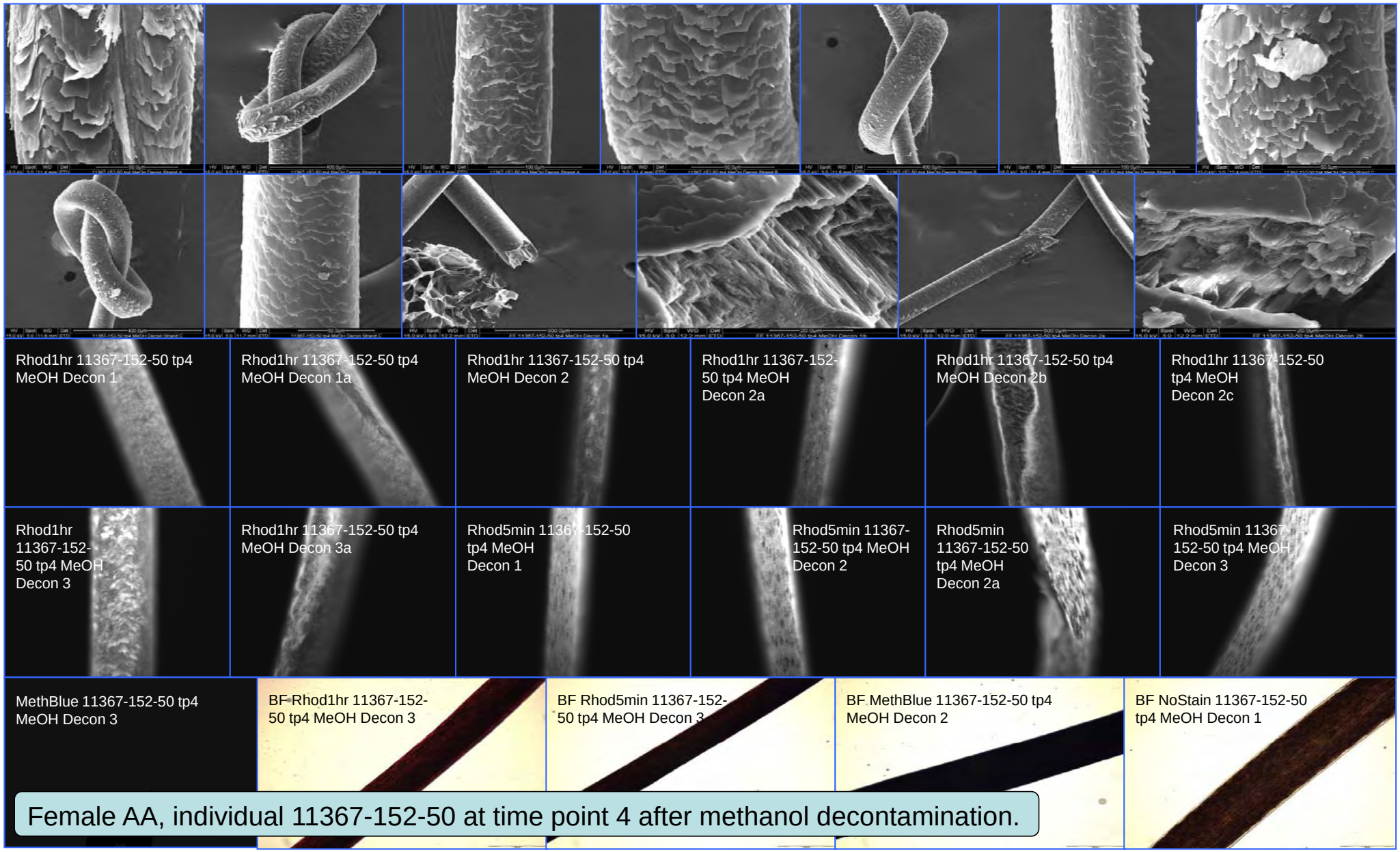

Rhod1hr 11367-152-37 tp4 MeOH Decon 1
Rhod1hr 11367-152-37 tp4 MeOH Decon 2
Rhod1hr 11367-152-37 tp4 MeOH Decon 3

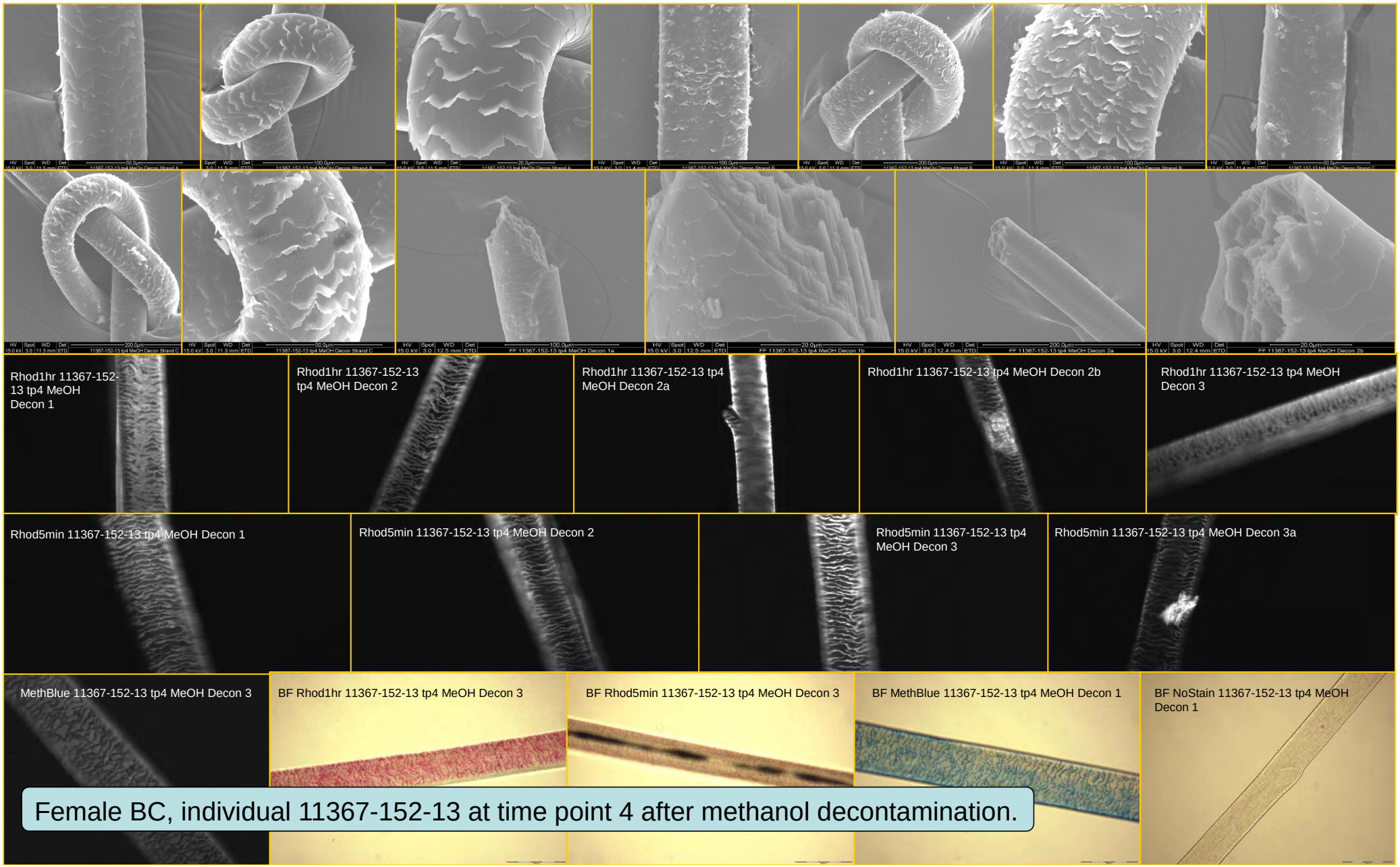

Rhod5min 11367-152-37 tp4 MeOH Decon 1
Rhod5min 11367-152-37 tp4 MeOH Decon 2
Rhod5min 11367-152-37 tp4 MeOH Decon 3

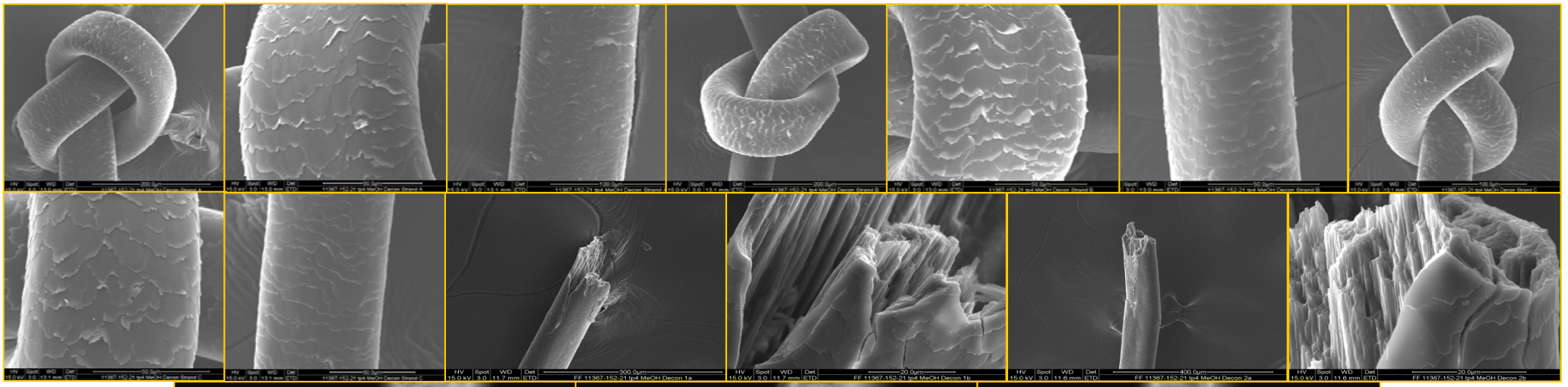

MethBlue 11367-152-37 tp4 MeOH Decon 2
BF Rhod1hr 11367-152-37 tp4 MeOH Decon 2
BF Rhod5min 11367-152-37 tp4 MeOH Decon 1
BF MethBlue 11367-152-37 tp4 MeOH Decon 1
BF NoStain 11367-152-37 tp4 MeOH Decon 1
Male AA, individual 11367-152-37 at time point 4 after methanol decontamination.

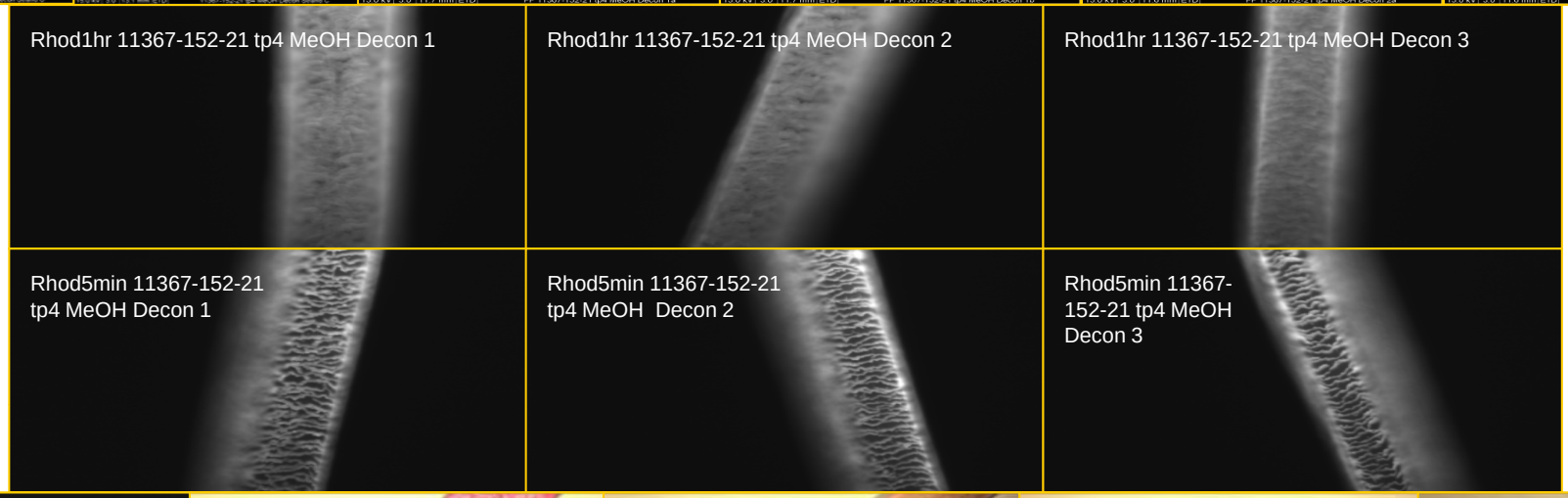

Rhod1hr 11367-152-45 tp4 MeOH Decon 1
Rhod1hr 11367-152-45 tp4 MeOH Decon 1a
Rhod1hr 11367-152-45 tp4 MeOH Decon 2
Rhod1hr 11367-152-45 tp4 MeOH Decon 3
Rhod1hr 11367-152-45 tp4 MeOH Decon 3a
Rhod5min 11367-152-45 tp4 MeOH Decon 1
Rhod5min 11367-152-45 tp4 MeOH Decon 2
Rhod5min 11367-152-45 tp4 MeOH Decon 2a
Rhod5min 11367-152-45 tp4 MeOH Decon 3
Rhod5min 11367-152-45 tp4 MeOH Decon 3a
Rhod5min 11367-152-45 tp4 MeOH Decon 3b
BF Rhod1hr 11367-152-45 tp4 MeOH Decon 3
BF Rhod5min 11367-152-45 tp4 MeOH Decon 1
BF MethBlue 11367-152-45 tp4 MeOH Decon 2
BF NoStain 11367-152-45 tp4 MeOH Decon 1
Female AA, individual 11367-152-45 at time point 4 after methanol decontamination.

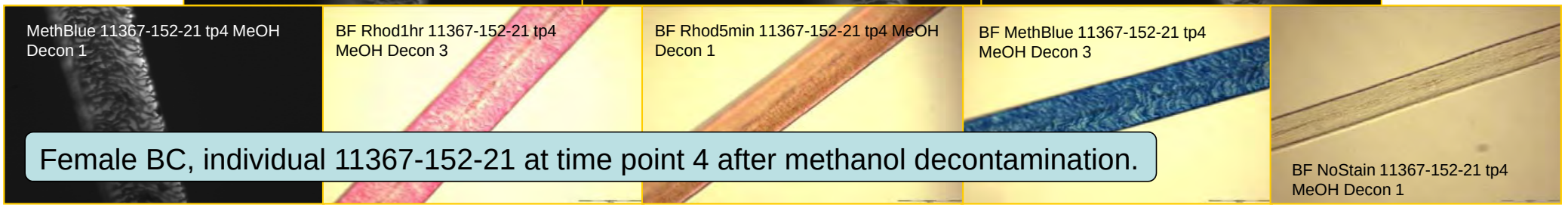

Rhod1hr 11367-152-49 tp4 MeOH Decon 1
Rhod1hr 11367-152-49 tp4 MeOH Decon 1a
Rhod1hr 11367-152-49 tp4 MeOH Decon 2
Rhod1hr 11367-152-49 tp4 MeOH Decon 3
Rhod5min 11367-152-49 tp4 MeOH Decon 1
Rhod5min 11367-152-49 tp4 MeOH Decon 1a
Rhod5min 11367-152-49 tp4 MeOH Decon 2
Rhod5min 11367-152-49 tp4 MeOH Decon 2a
Rhod5min 11367-152-49 tp4 MeOH Decon 3
MethBlue 11367-152-49 tp4 MeOH Decon 3
BF Rhod1hr 11367-152-49 tp4 MeOH Decon 3
BF Rhod5min 11367-152-49 tp4 MeOH Decon 3
BF MethBlue 11367-152-49 tp4 MeOH Decon 3
BF NoStain 11367-152-49 tp4 MeOH Decon 1
Male AA, individual 11367-152-49 at time point 4 after methanol decontamination.

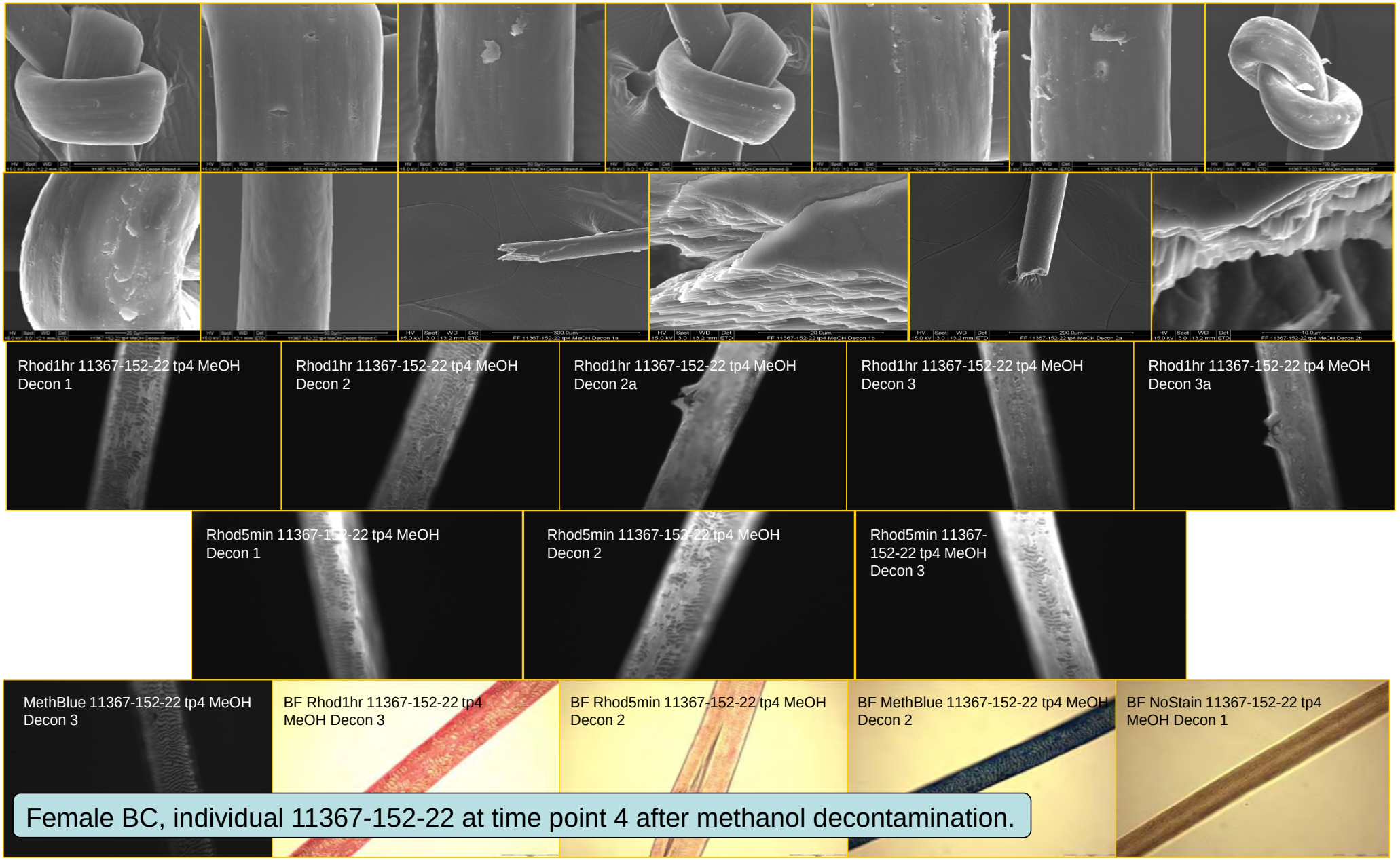

Rhod1hr 11367-152-50 tp4 MeOH Decon 1
Rhod1hr 11367-152-50 tp4 MeOH Decon 1a
Rhod1hr 11367-152-50 tp4 MeOH Decon 2
Rhod1hr 11367-152-50 tp4 MeOH Decon 2a
Rhod1hr 11367-152-50 tp4 MeOH Decon 2b
Rhod1hr 11367-152-50 tp4 MeOH Decon 2c
Rhod1hr 11367-152-50 tp4 MeOH Decon 3
Rhod1hr 11367-152-50 tp4 MeOH Decon 3a
Rhod5min 11367-152-50 tp4 MeOH Decon 1
Rhod5min 11367-152-50 tp4 MeOH Decon 2
Rhod5min 11367-152-50 tp4 MeOH Decon 2a
Rhod5min 11367-152-50 tp4 MeOH Decon 3
MethBlue 11367-152-50 tp4 MeOH Decon 3
BF Rhod1hr 11367-152-50 tp4 MeOH Decon 3
BF Rhod5min 11367-152-50 tp4 MeOH Decon 3
BF MethBlue 11367-152-50 tp4 MeOH Decon 2
BF NoStain 11367-152-50 tp4 MeOH Decon 1
Female AA, individual 11367-152-50 at time point 4 after methanol decontamination.

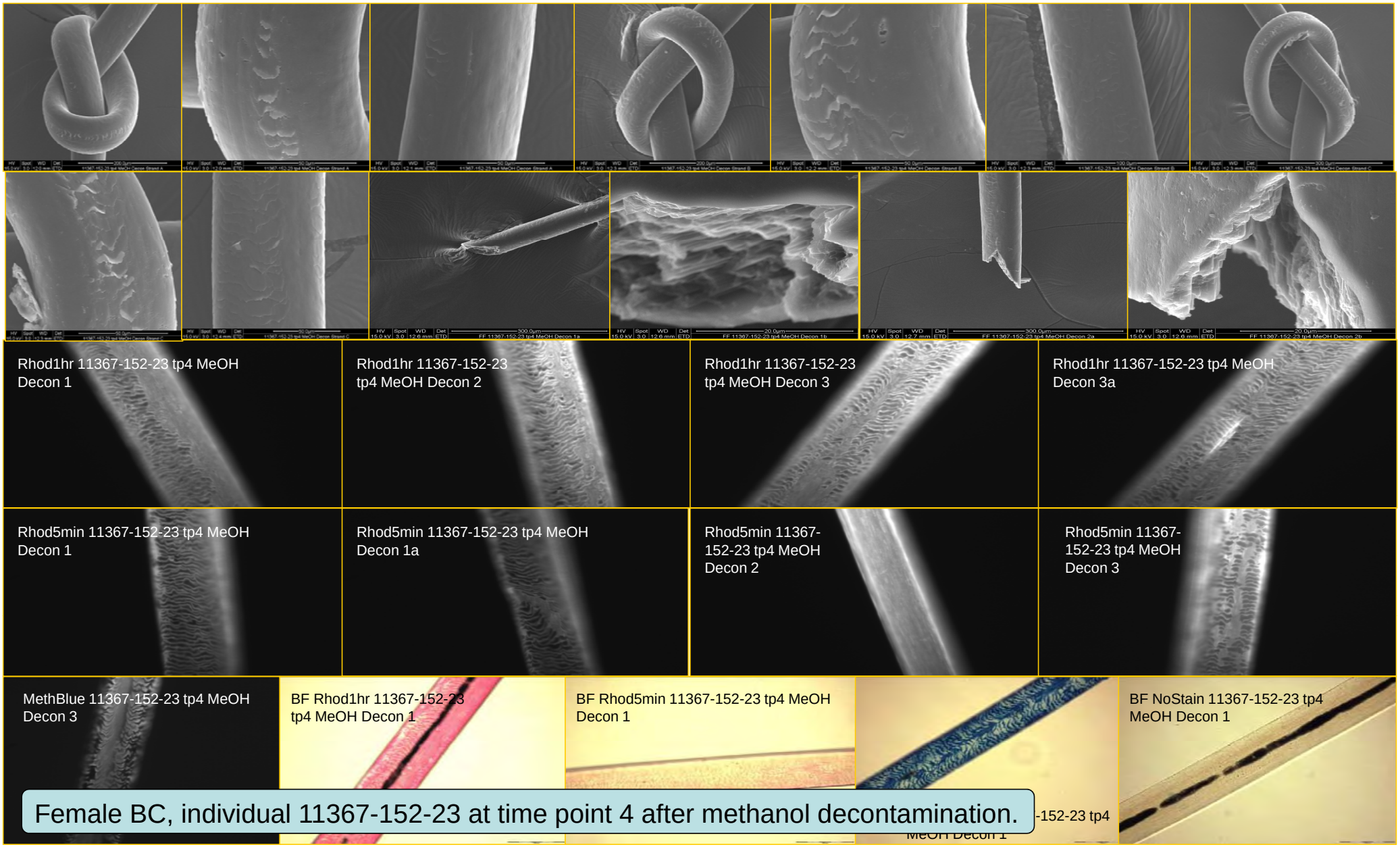

Rhod1hr 11367-152-13 tp4 MeOH Decon 1
Rhod1hr 11367-152-13 tp4 MeOH Decon 2
Rhod1hr 11367-152-13 tp4 MeOH Decon 2a
Rhod1hr 11367-152-13 tp4 MeOH Decon 2b
Rhod1hr 11367-152-13 tp4 MeOH Decon 3
Rhod5min 11367-152-13 tp4 MeOH Decon 1
Rhod5min 11367-152-13 tp4 MeOH Decon 2
Rhod5min 11367-152-13 tp4 MeOH Decon 3
Rhod5min 11367-152-13 tp4 MeOH Decon 3a
MethBlue 11367-152-13 tp4 MeOH Decon 3
BF Rhod1hr 11367-152-13 tp4 MeOH Decon 3
BF Rhod5min 11367-152-13 tp4 MeOH Decon 3
BF MethBlue 11367-152-13 tp4 MeOH Decon 1
BF NoStain 11367-152-13 tp4 MeOH Decon 1
Female BC, individual 11367-152-13 at time point 4 after methanol decontamination.

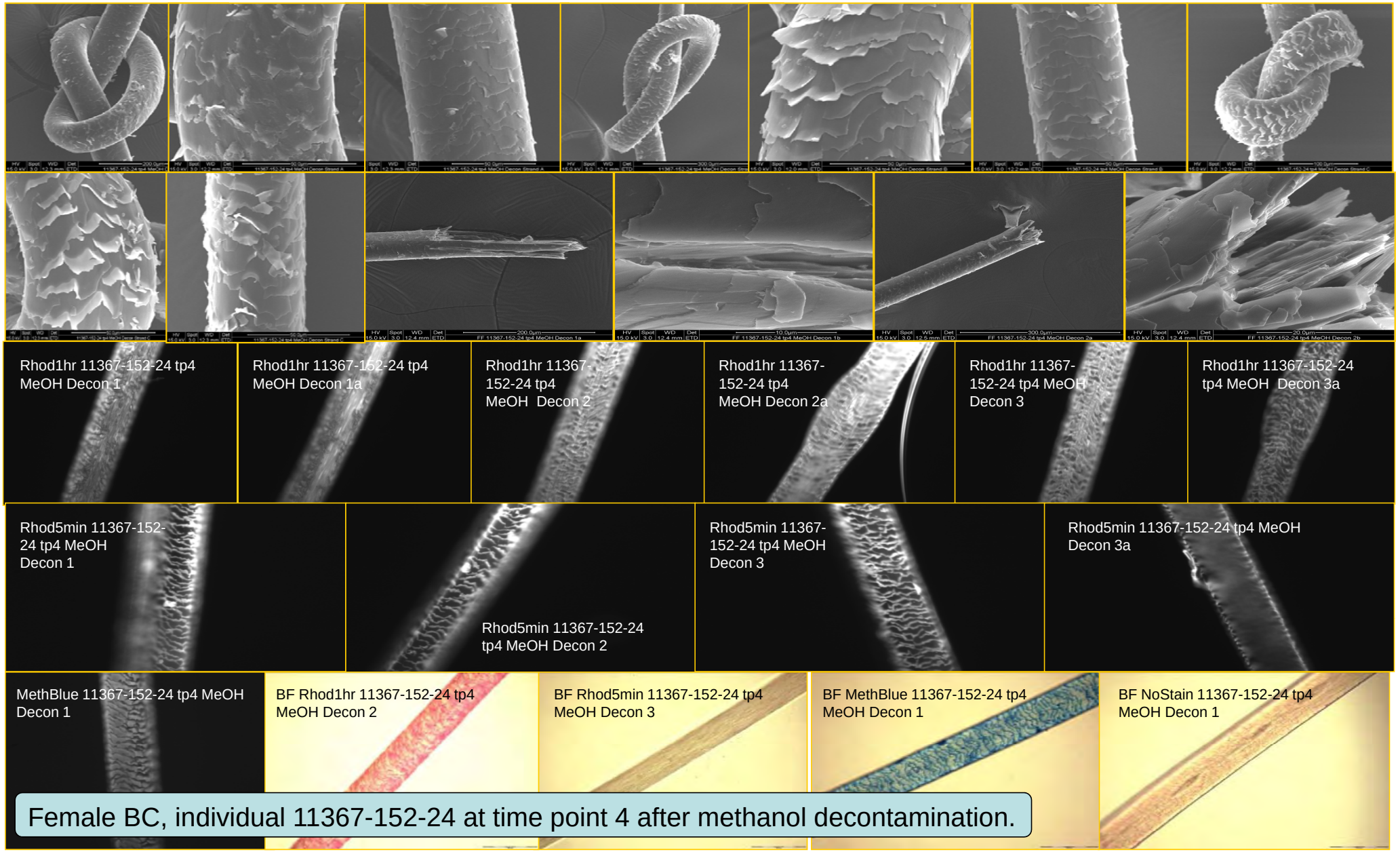


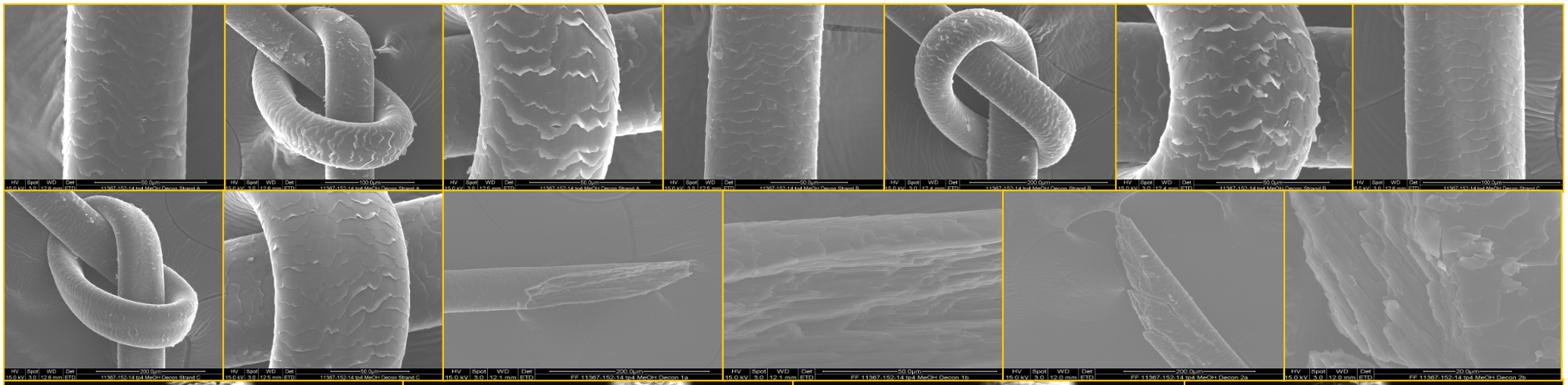

Rhod1hr 11367-152-21 tp4 MeOH Decon 1
Rhod1hr 11367-152-21 tp4 MeOH Decon 2
Rhod1hr 11367-152-21 tp4 MeOH Decon 3
Rhod5min 11367-152-21 tp4 MeOH Decon 1
Rhod5min 11367-152-21 tp4 MeOH Decon 2
Rhod5min 11367-152-21 tp4 MeOH Decon 3

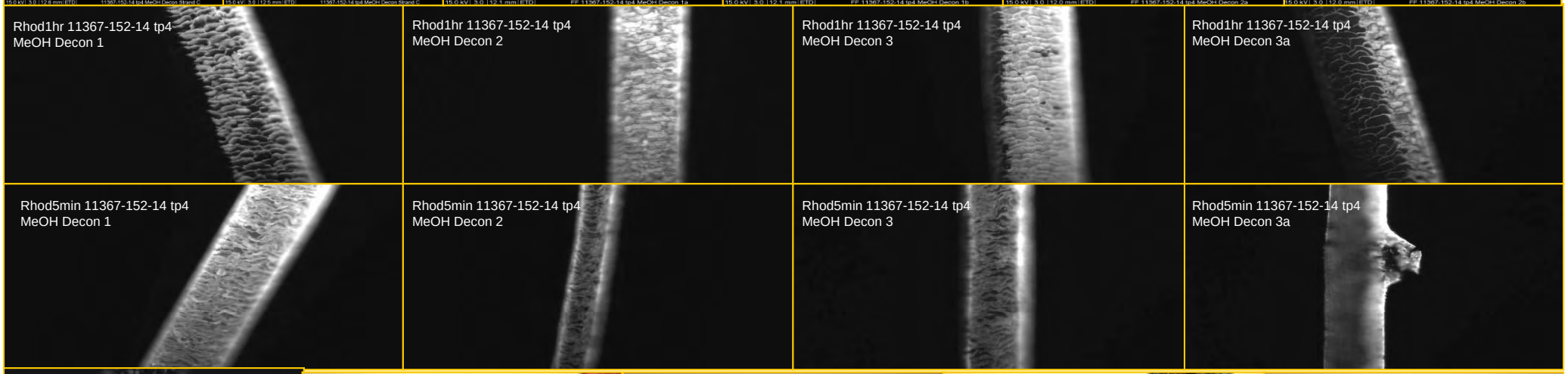

MethBlue 11367-152-21 tp4 MeOH Decon 1
BF Rhod1hr 11367-152-21 tp4 MeOH Decon 3
BF Rhod5min 11367-152-21 tp4 MeOH Decon 1
BF MethBlue 11367-152-21 tp4 MeOH Decon 3
BF NoStain 11367-152-21 tp4 MeOH Decon 1
Female BC, individual 11367-152-21 at time point 4 after methanol decontamination.

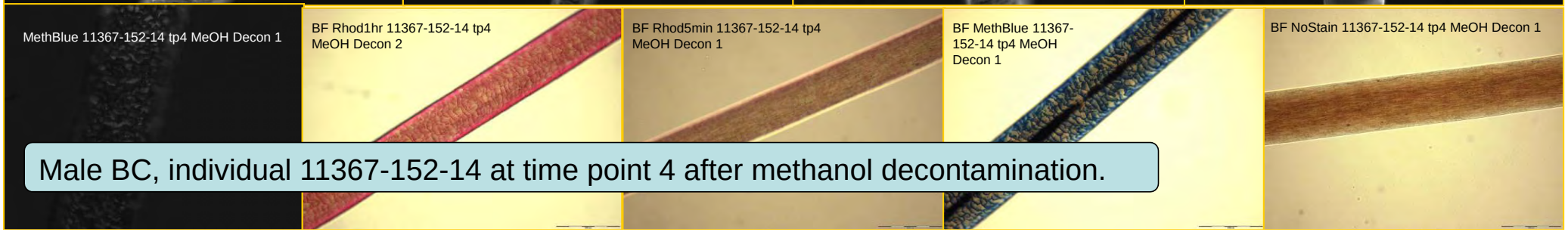

Rhod1hr 11367-152-22 tp4 MeOH Decon 1
Rhod1hr 11367-152-22 tp4 MeOH Decon 2
Rhod1hr 11367-152-22 tp4 MeOH Decon 2a
Rhod1hr 11367-152-22 tp4 MeOH Decon 3
Rhod1hr 11367-152-22 tp4 MeOH Decon 3a
Rhod5min 11367-152-22 tp4 MeOH Decon 1
Rhod5min 11367-152-22 tp4 MeOH Decon 2
Rhod5min 11367-152-22 tp4 MeOH Decon 3
MethBlue 11367-152-22 tp4 MeOH Decon 3
BF Rhod1hr 11367-152-22 tp4 MeOH Decon 3
BF Rhod5min 11367-152-22 tp4 MeOH Decon 2
BF MethBlue 11367-152-22 tp4 MeOH Decon 2
BF NoStain 11367-152-22 tp4 MeOH Decon 1
Female BC, individual 11367-152-22 at time point 4 after methanol decontamination.


Rhod1hr 11367-152-23 tp4 MeOH Decon 1
Rhod1hr 11367-152-23 tp4 MeOH Decon 2
Rhod1hr 11367-152-23 tp4 MeOH Decon 3
Rhod1hr 11367-152-23 tp4 MeOH Decon 3a
Rhod5min 11367-152-23 tp4 MeOH Decon 1
Rhod5min 11367-152-23 tp4 MeOH Decon 1a
Rhod5min 11367-152-23 tp4 MeOH Decon 2
Rhod5min 11367-152-23 tp4 MeOH Decon 3
MethBlue 11367-152-23 tp4 MeOH Decon 3
BF Rhod1hr 11367-152-23 tp4 MeOH Decon 1
BF Rhod5min 11367-152-23 tp4 MeOH Decon 1
BF NoStain 11367-152-23 tp4 MeOH Decon 1
Female BC, individual 11367-152-23 at time point 4 after methanol decontamination.

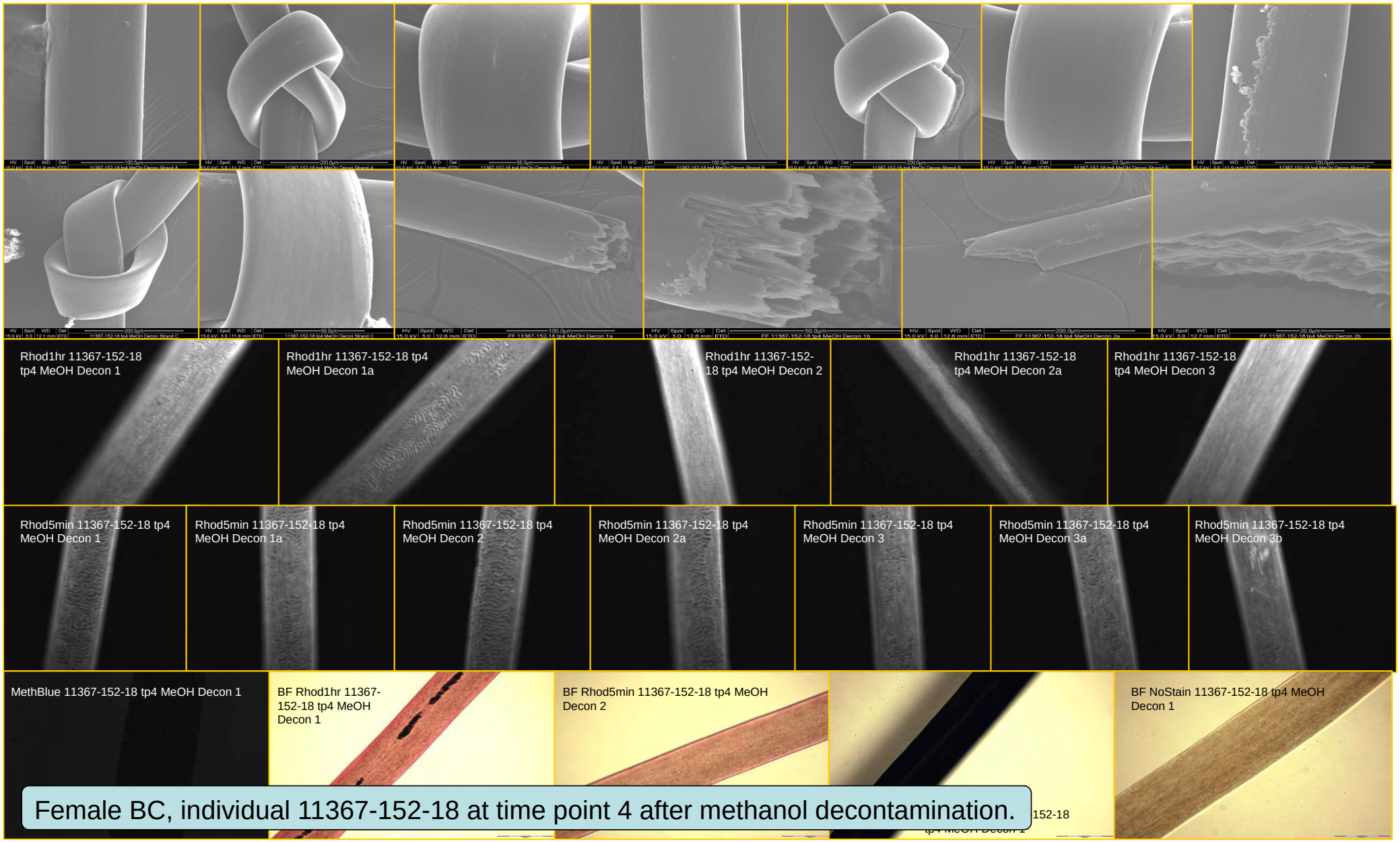

Rhod1hr 11367-152-24 tp4 MeOH Decon 1
Rhod1hr 11367-152-24 tp4 MeOH Decon 1a
Rhod1hr 11367-152-24 tp4 MeOH Decon 2
Rhod1hr 11367-152-24 tp4 MeOH Decon 2a
Rhod1hr 11367-152-24 tp4 MeOH Decon 3
Rhod1hr 11367-152-24 tp4 MeOH Decon 3a
Rhod5min 11367-152-24 tp4 MeOH Decon 1
Rhod5min 11367-152-24 tp4 MeOH Decon 2
Rhod5min 11367-152-24 tp4 MeOH Decon 3
Rhod5min 11367-152-24 tp4 MeOH Decon 3a
MethBlue 11367-152-24 tp4 MeOH Decon 1
BF Rhod1hr 11367-152-24 tp4 MeOH Decon 2
BF Rhod5min 11367-152-24 tp4 MeOH Decon 3
BF MethBlue 11367-152-24 tp4 MeOH Decon 1
BF NoStain 11367-152-24 tp4 MeOH Decon 1
Female BC, individual 11367-152-24 at time point 4 after methanol decontamination.

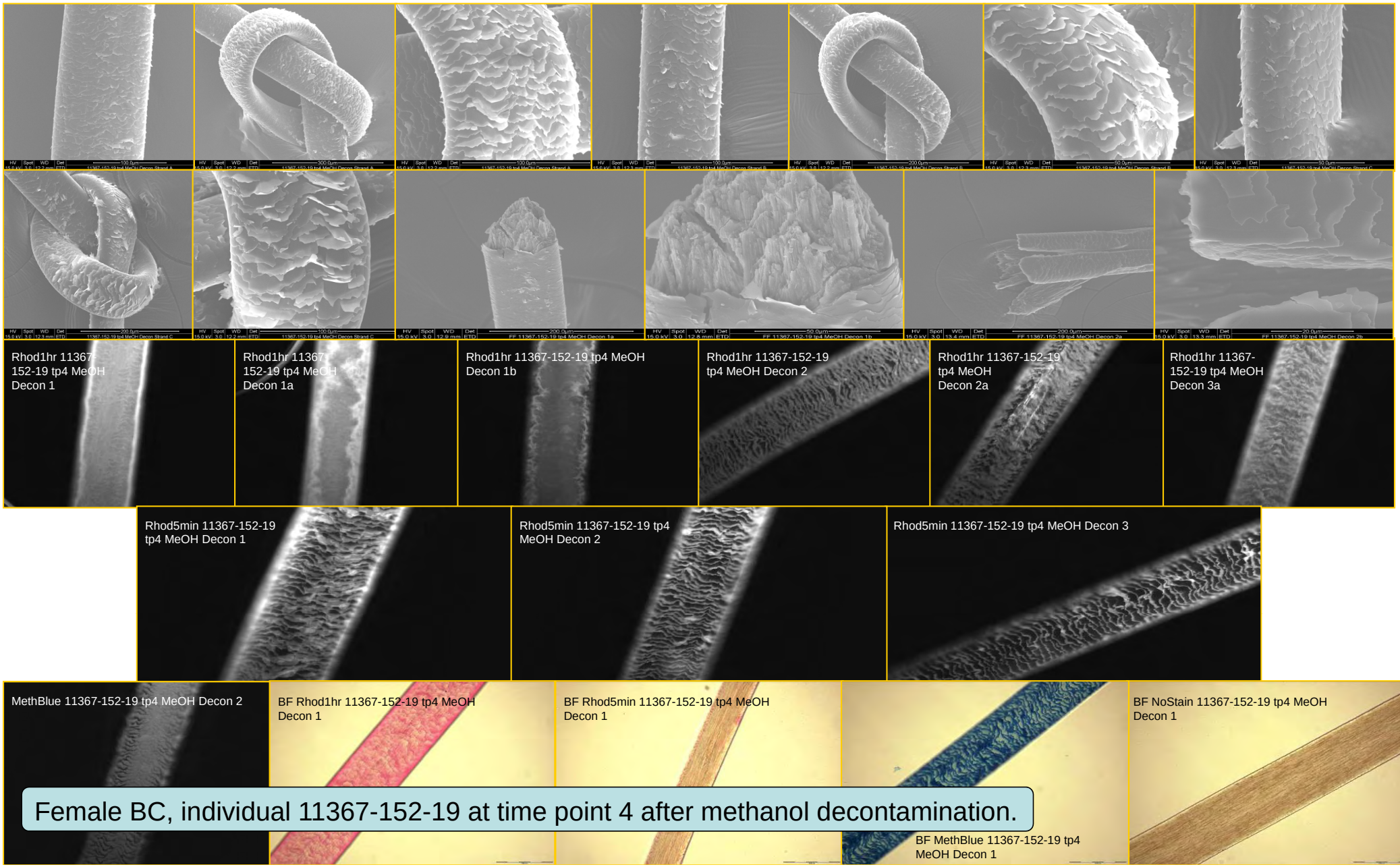


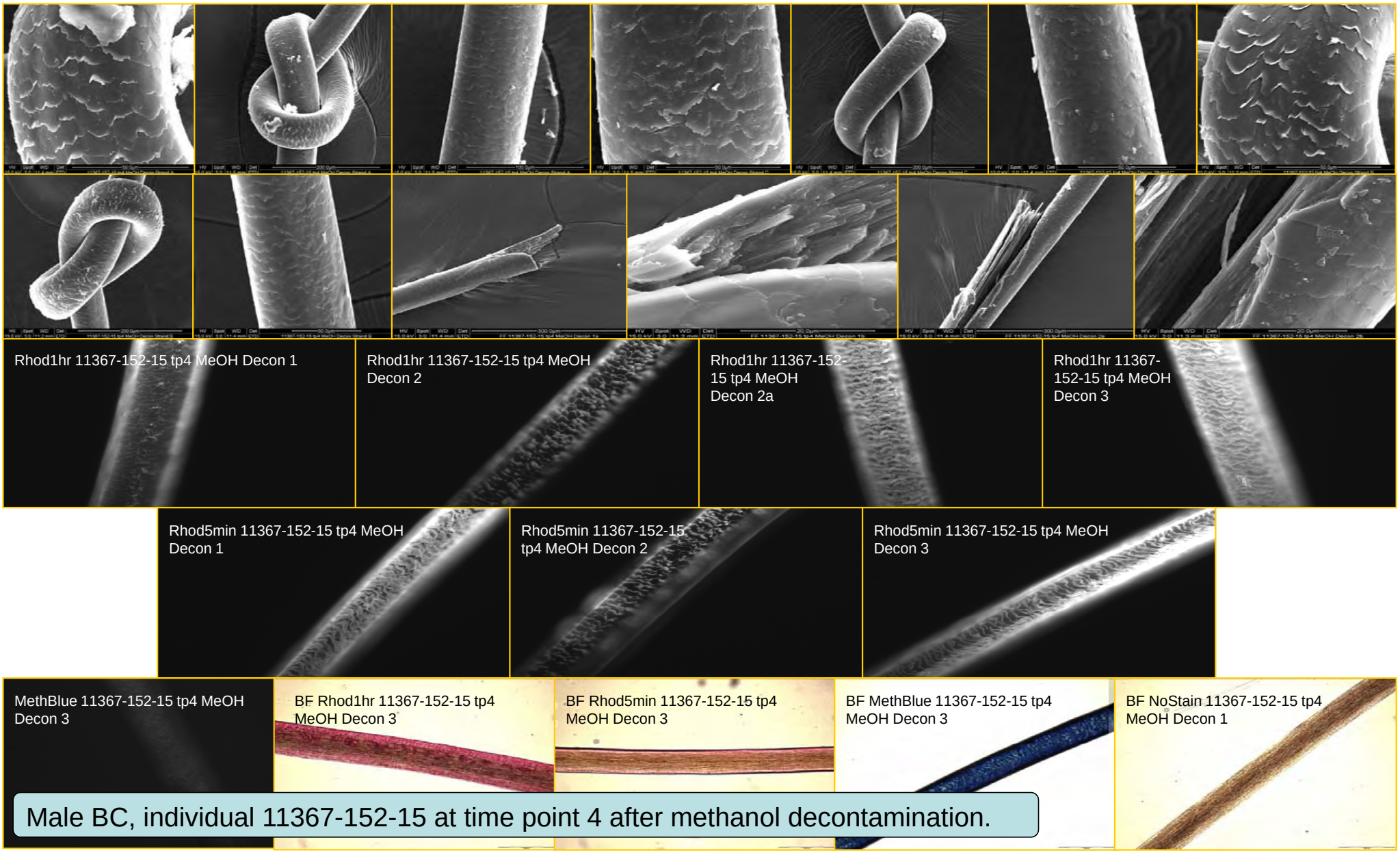




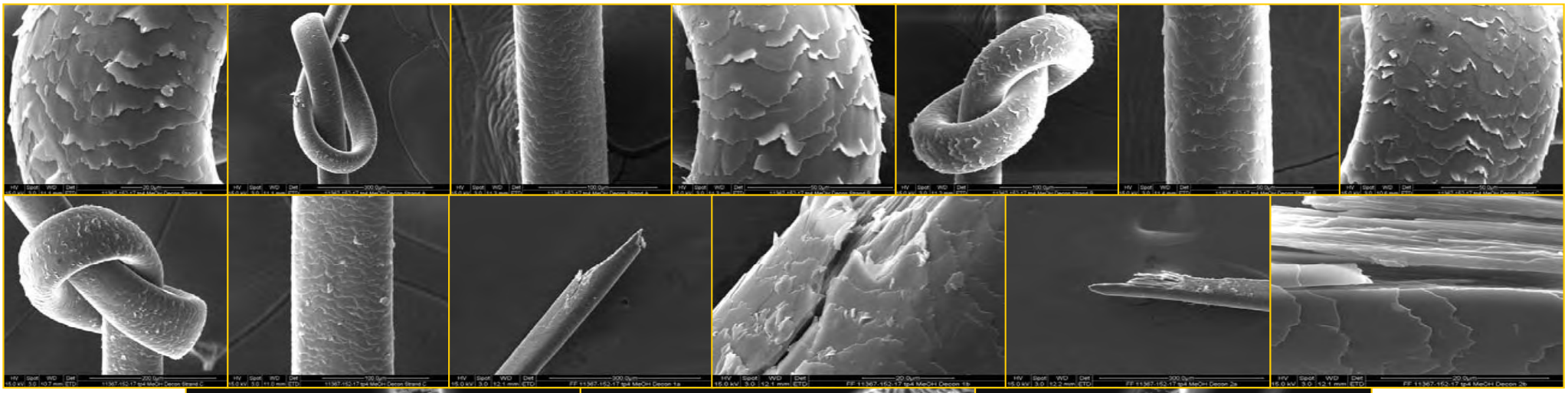
Male BC, individual 11367-152-14 at time point 4 after methanol decontamination.

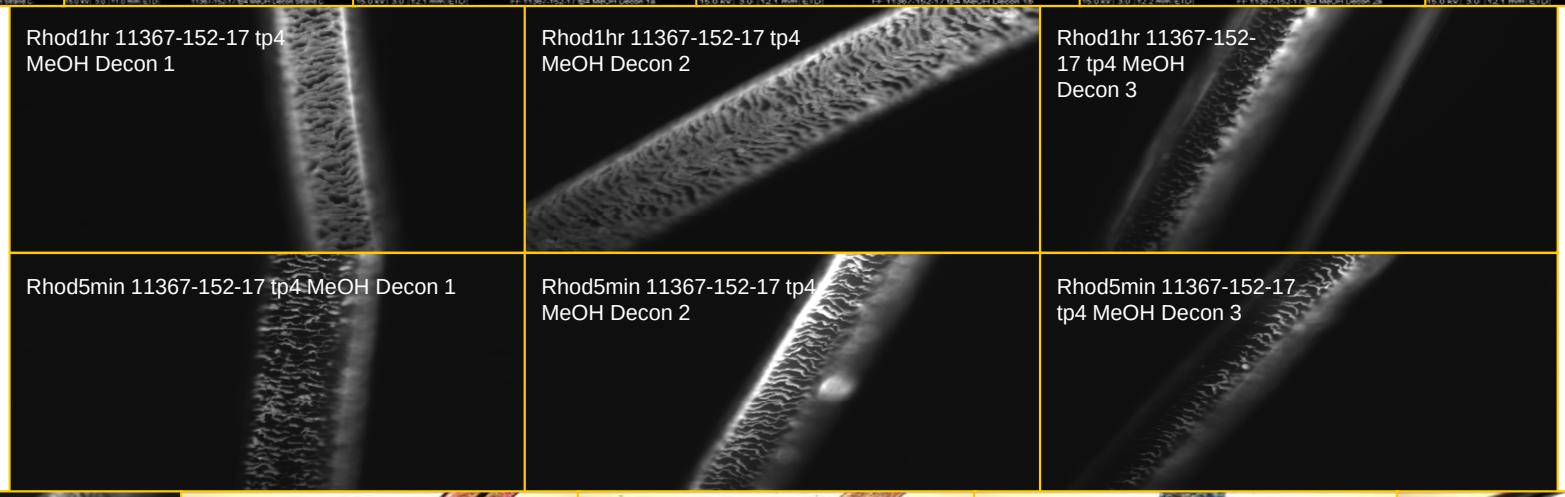

Rhod1hr 11367-152-18 tp4 MeOH Decon 1
Rhod1hr 11367-152-18 tp4 MeOH Decon 1a
Rhod1hr 11367-152-18 tp4 MeOH Decon 2
Rhod1hr 11367-152-18 tp4 MeOH Decon 2a
Rhod1hr 11367-152-18 tp4 MeOH Decon 3
Rhod5min 11367-152-18 tp4 MeOH Decon 1
Rhod5min 11367-152-18 tp4 MeOH Decon 1a
Rhod5min 11367-152-18 tp4 MeOH Decon 2
Rhod5min 11367-152-18 tp4 MeOH Decon 2a
Rhod5min 11367-152-18 tp4 MeOH Decon 3
Rhod5min 11367-152-18 tp4 MeOH Decon 3a
Rhod5min 11367-152-18 tp4 MeOH Decon 3b
MethBlue 11367-152-18 tp4 MeOH Decon 1
BF Rhod1hr 11367-152-18 tp4 MeOH Decon 1
BF Rhod5min 11367-152-18 tp4 MeOH Decon 2
BF NoStain 11367-152-18 tp4 MeOH Decon 1
Female BC, individual 11367-152-18 at time point 4 after methanol decontamination.

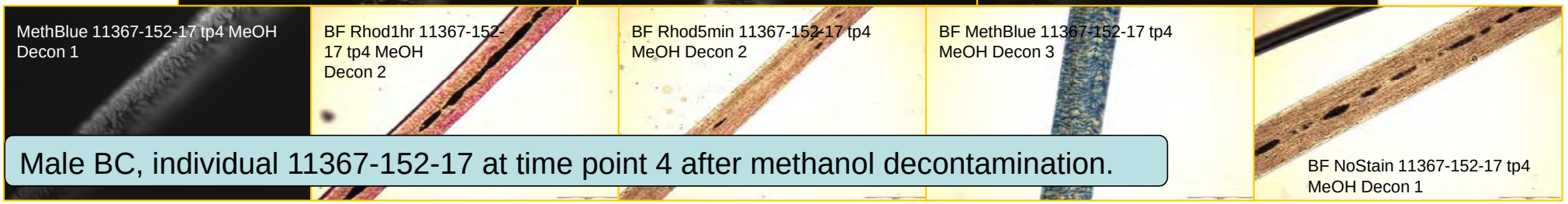

Rhod1hr 11367-152-19 tp4 MeOH Decon 1
Rhod1hr 11367-152-19 tp4 MeOH Decon 1a
Rhod1hr 11367-152-19 tp4 MeOH Decon 1b
Rhod1hr 11367-152-19 tp4 MeOH Decon 2
Rhod1hr 11367-152-19 tp4 MeOH Decon 2a
Rhod1hr 11367-152-19 tp4 MeOH Decon 3a
Rhod5min 11367-152-19 tp4 MeOH Decon 1
Rhod5min 11367-152-19 tp4 MeOH Decon 2
Rhod5min 11367-152-19 tp4 MeOH Decon 3
MethBlue 11367-152-19 tp4 MeOH Decon 2
BF Rhod1hr 11367-152-19 tp4 MeOH Decon 1
BF Rhod5min 11367-152-19 tp4 MeOH Decon 1
BF MethBlue 11367-152-19 tp4 MeOH Decon 1
BF NoStain 11367-152-19 tp4 MeOH Decon 1
Female BC, individual 11367-152-19 at time point 4 after methanol decontamination.


Rhod1hr 11367-152-15 tp4 MeOH Decon 1
Rhod1hr 11367-152-15 tp4 MeOH Decon 2
Rhod1hr 11367-152-15 tp4 MeOH Decon 2a
Rhod1hr 11367-152-15 tp4 MeOH Decon 3
Rhod5min 11367-152-15 tp4 MeOH Decon 1
Rhod5min 11367-152-15 tp4 MeOH Decon 2
Rhod5min 11367-152-15 tp4 MeOH Decon 3
MethBlue 11367-152-15 tp4 MeOH Decon 3
BF Rhod1hr 11367-152-15 tp4 MeOH Decon 3
BF Rhod5min 11367-152-15 tp4 MeOH Decon 3
BF MethBlue 11367-152-15 tp4 MeOH Decon 3
BF NoStain 11367-152-15 tp4 MeOH Decon 1
Male BC, individual 11367-152-15 at time point 4 after methanol decontamination.

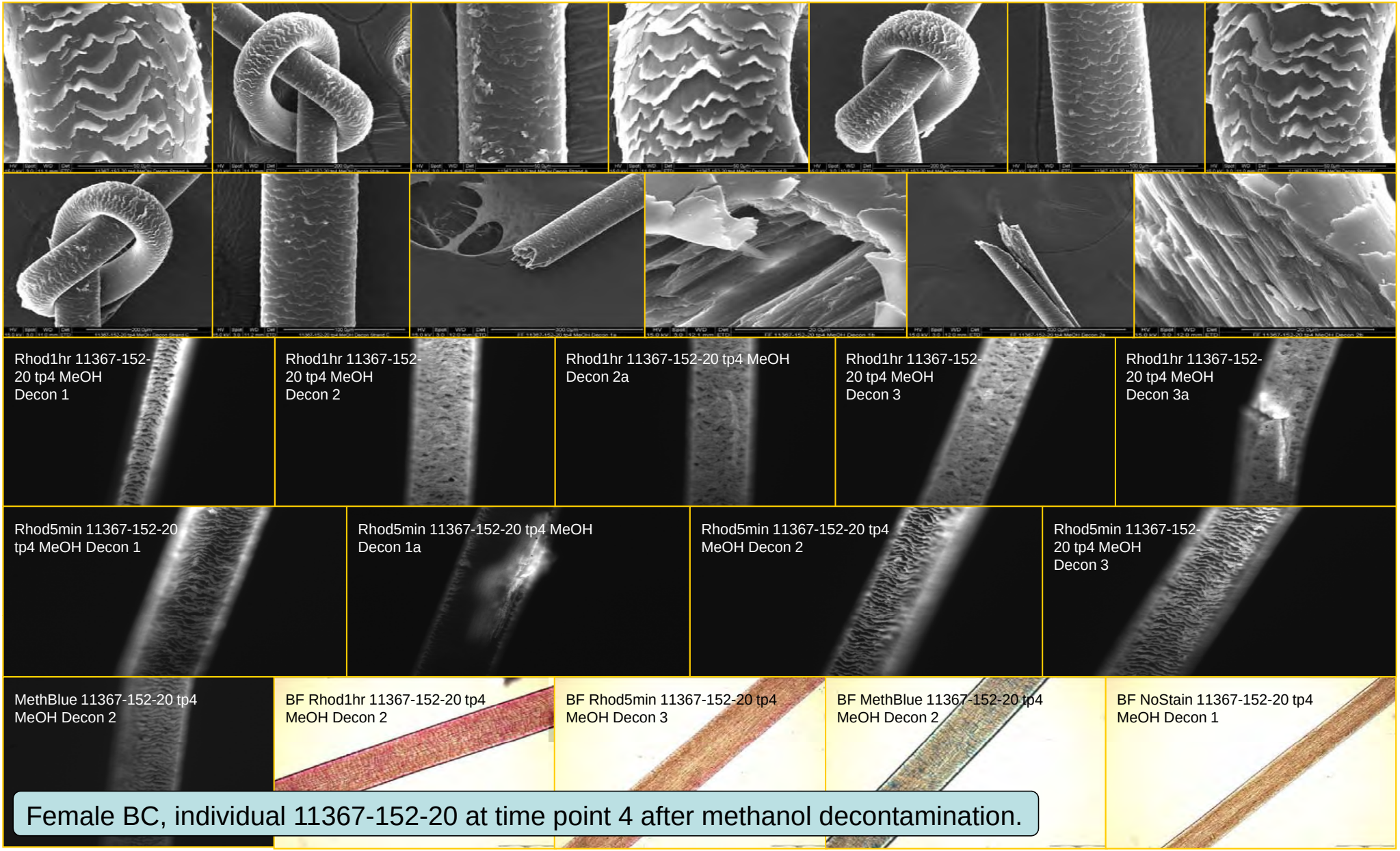

Rhod1hr 11367-152-16 tp4 MeOH Decon 1
Rhod1hr 11367-152-16 tp4 MeOH Decon 1a
Rhod1hr 11367-152-16 tp4 MeOH Decon 2
Rhod1hr 11367-152-16 tp4 MeOH Decon 2a
Rhod1hr 11367-152-16 tp4 MeOH Decon 3
Rhod1hr 11367-152-16 tp4 MeOH Decon 3a
Rhod5min 11367-152-16 tp4 MeOH Decon 1
Rhod5min 11367-152-16 tp4 MeOH Decon 1a
Rhod5min 11367-152-16 tp4 MeOH Decon 2
Rhod5min 11367-152-16 tp4 MeOH Decon 3
MethBlue 11367-152-16 tp4 MeOH Decon 2
BF Rhod1hr 11367-152-16 tp4 MeOH Decon 1
BF Rhod5min 11367-152-16 tp4 MeOH Decon 1
BF MethBlue 11367-152-16 tp4 MeOH Decon 3
BF NoStain 11367-152-16 tp4 MeOH Decon 1
Male BC, individual 11367-152-16 at time point 4 after methanol decontamination.

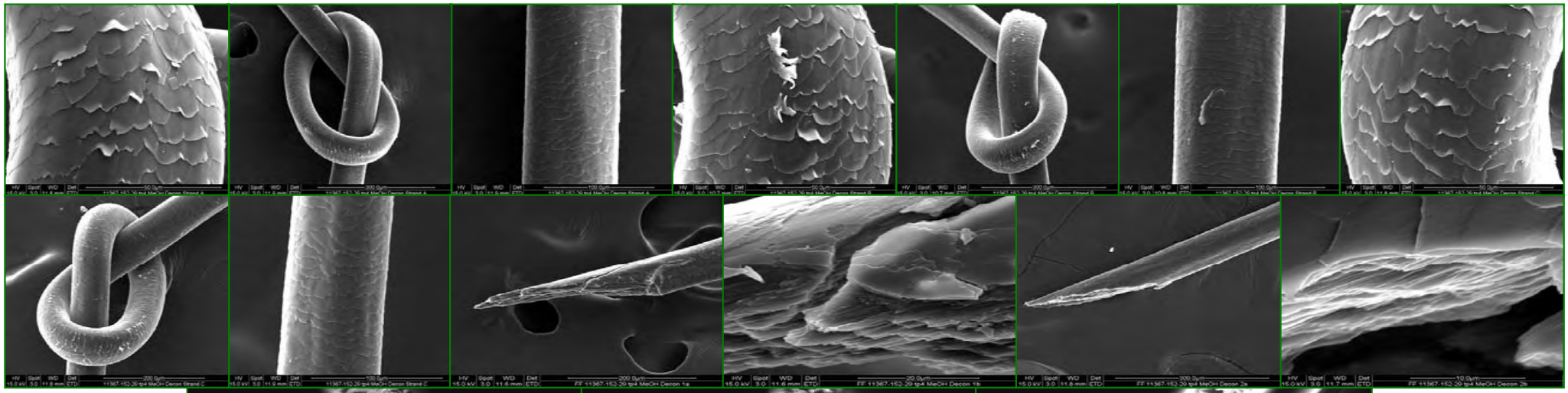


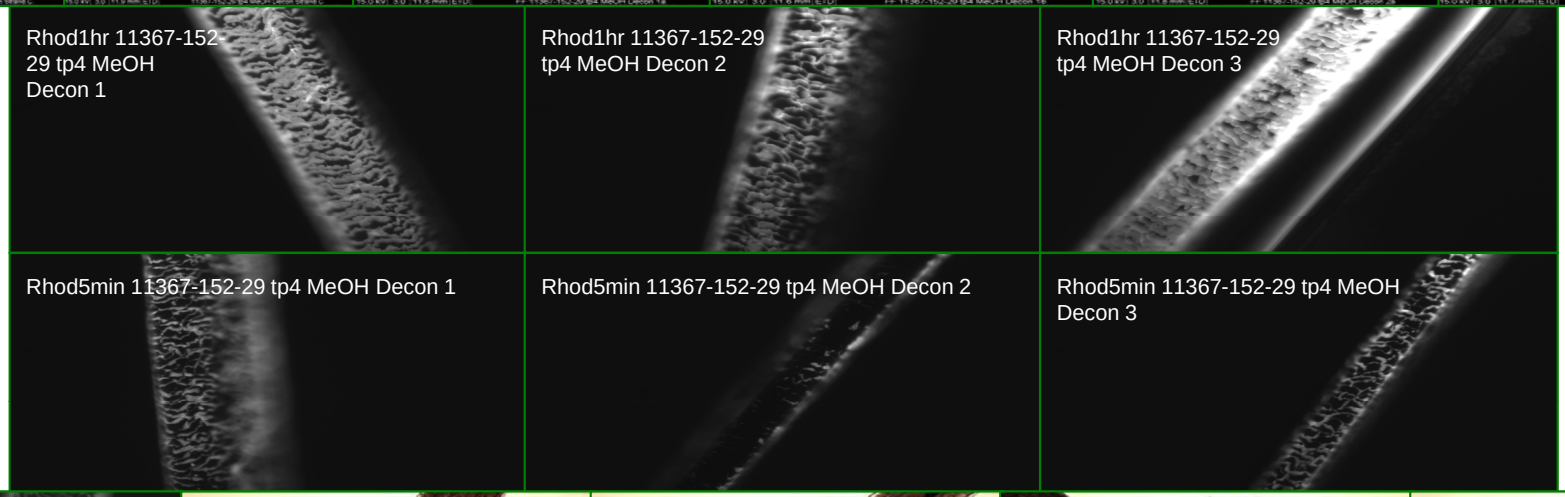


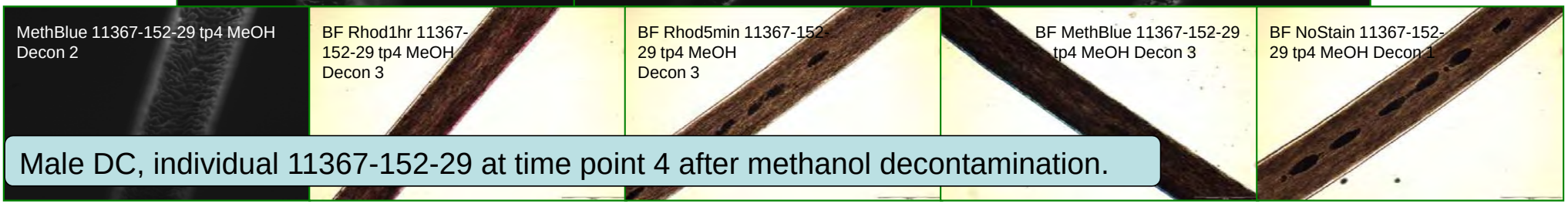


Male BC, individual 11367-152-17 at time point 4 after methanol decontamination.

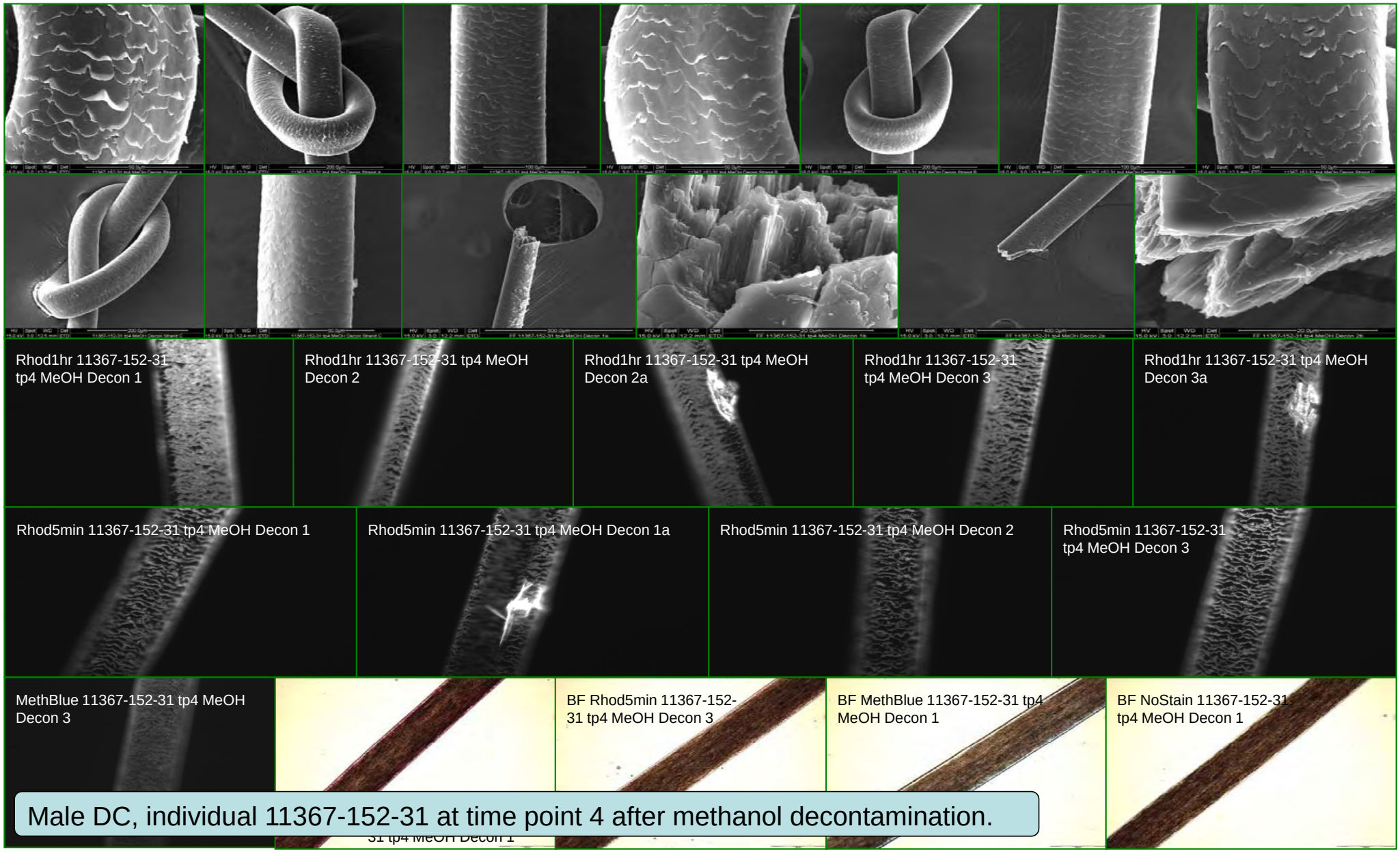

Rhod1hr 11367-152-20 tp4 MeOH Decon 1
Rhod1hr 11367-152-20 tp4 MeOH Decon 2
Rhod1hr 11367-152-20 tp4 MeOH Decon 2a
Rhod1hr 11367-152-20 tp4 MeOH Decon 3
Rhod1hr 11367-152-20 tp4 MeOH Decon 3a
Rhod5min 11367-152-20 tp4 MeOH Decon 1
Rhod5min 11367-152-20 tp4 MeOH Decon 1a
Rhod5min 11367-152-20 tp4 MeOH Decon 2
Rhod5min 11367-152-20 tp4 MeOH Decon 3
MethBlue 11367-152-20 tp4 MeOH Decon 2
BF Rhod1hr 11367-152-20 tp4 MeOH Decon 2
BF Rhod5min 11367-152-20 tp4 MeOH Decon 3
BF MethBlue 11367-152-20 tp4 MeOH Decon 2
BF NoStain 11367-152-20 tp4 MeOH Decon 1
Female BC, individual 11367-152-20 at time point 4 after methanol decontamination.



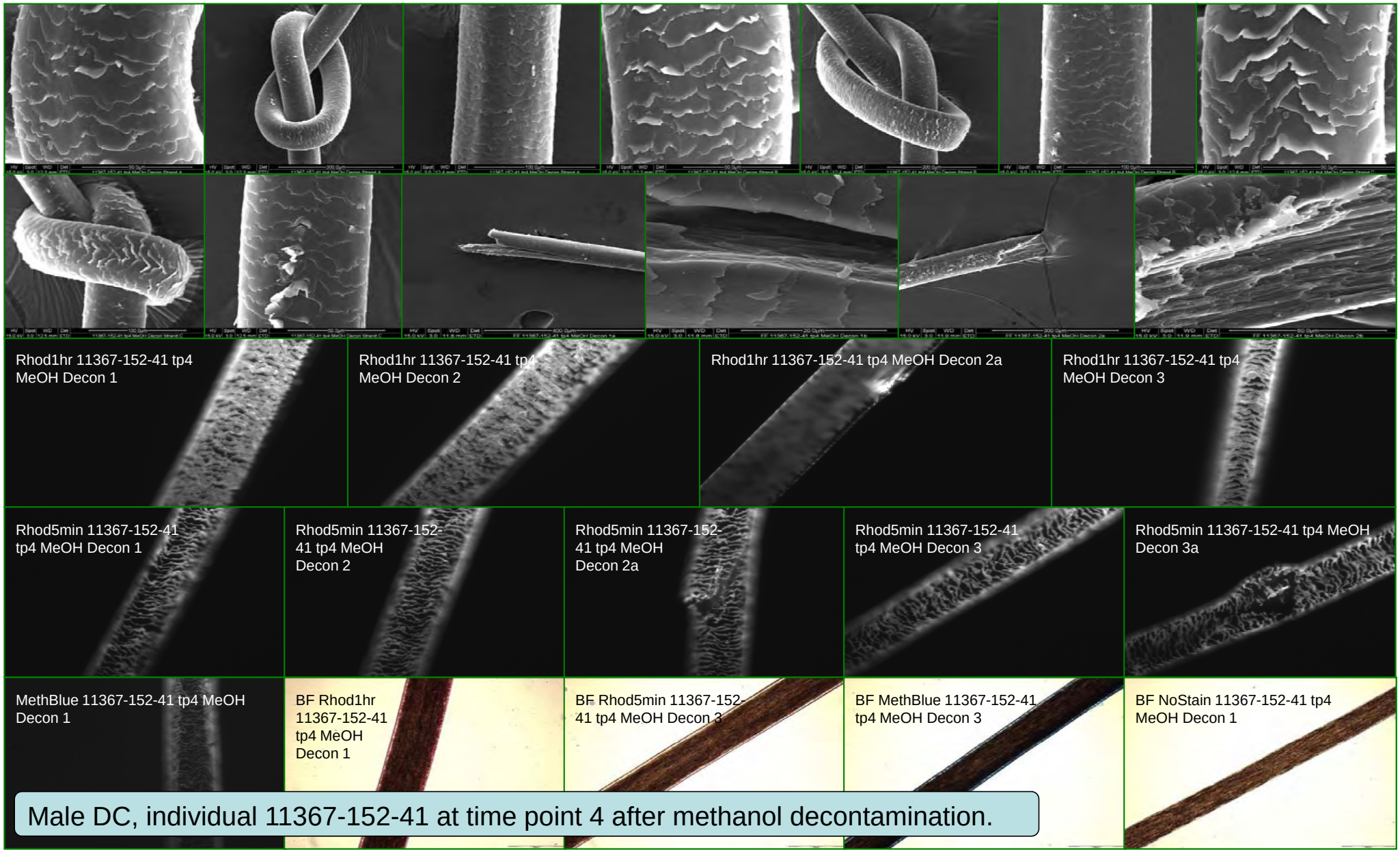


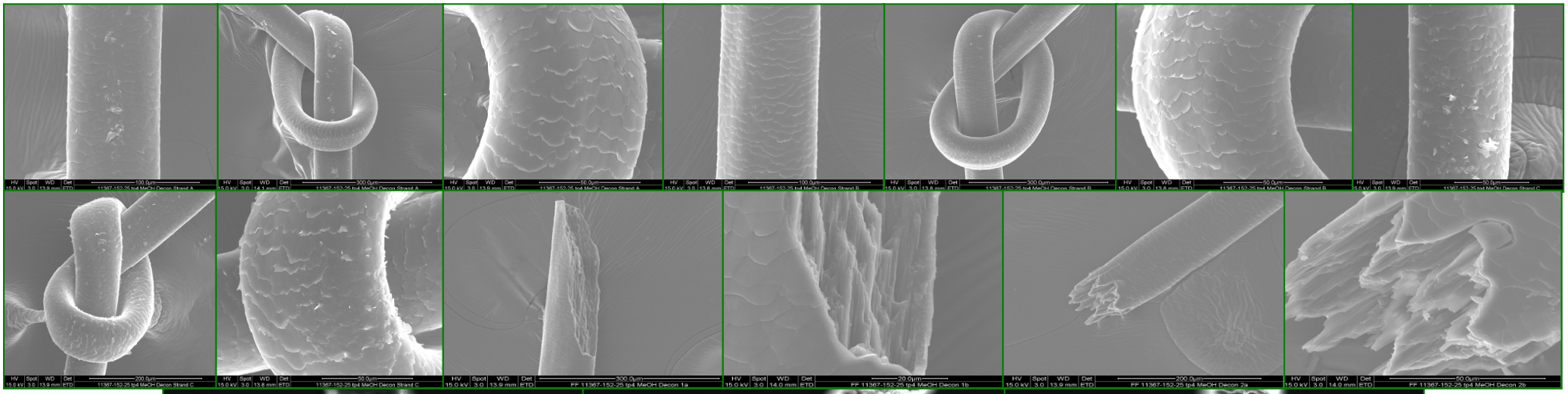


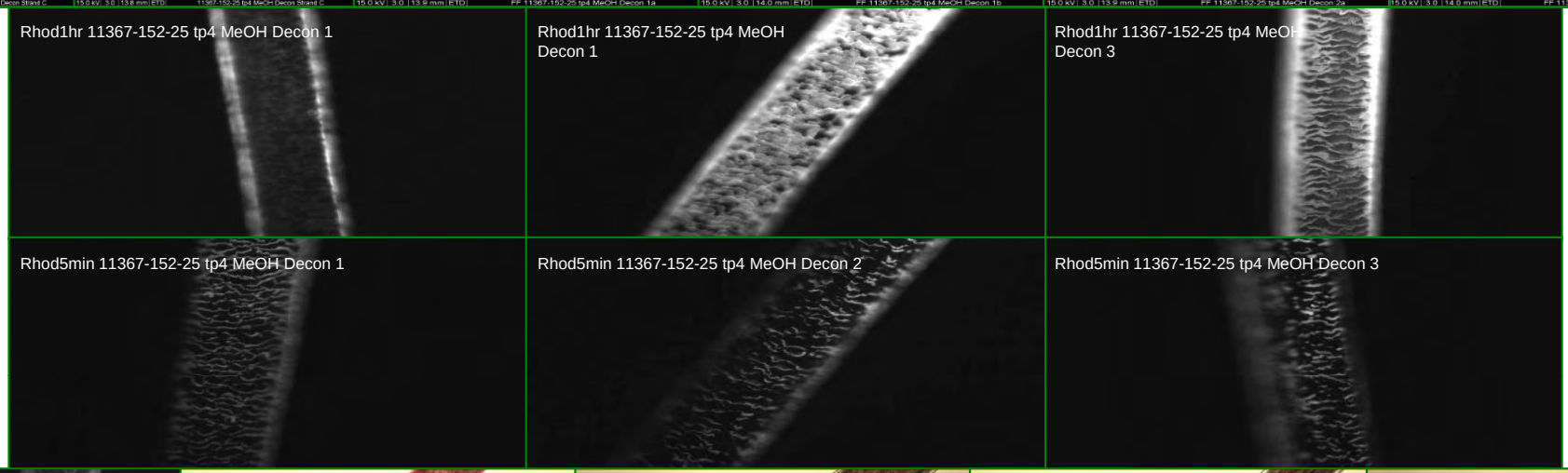
Male DC, individual 11367-152-29 at time point 4 after methanol decontamination.

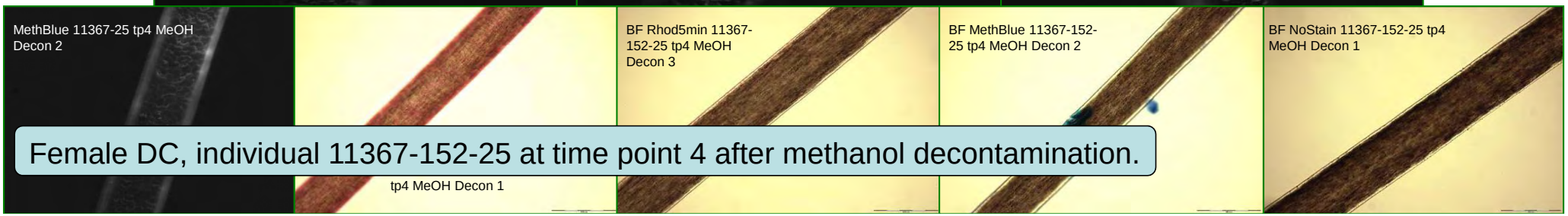

Rhod1hr 11367-152-31 tp4 MeOH Decon 1
Rhod1hr 11367-152-31 tp4 MeOH Decon 2
Rhod1hr 11367-152-31 tp4 MeOH Decon 2a
Rhod1hr 11367-152-31 tp4 MeOH Decon 3
Rhod1hr 11367-152-31 tp4 MeOH Decon 3a
Rhod5min 11367-152-31 tp4 MeOH Decon 1
Rhod5min 11367-152-31 tp4 MeOH Decon 1a
Rhod5min 11367-152-31 tp4 MeOH Decon 2
Rhod5min 11367-152-31 tp4 MeOH Decon 3
MethBlue 11367-152-31 tp4 MeOH Decon 3
BF Rhod5min 11367-152-31 tp4 MeOH Decon 3
BF MethBlue 11367-152-31 tp4 MeOH Decon 1
BF NoStain 11367-152-31 tp4 MeOH Decon 1
Male DC, individual 11367-152-31 at time point 4 after methanol decontamination.


Rhod1hr 11367-152-33 tp4 MeOH Decon 1
Rhod1hr 11367-152-33 tp4 MeOH Decon 1a
Rhod1hr 11367-152-33 tp4 MeOH Decon 2
Rhod1hr 11367-152-33 tp4 MeOH Decon 3
Rhod5min 11367-152-33 tp4 MeOH Decon 1
Rhod5min 11367-152-33 tp4 MeOH Decon 2
Rhod5min 11367-152-33 tp4 MeOH Decon 2a
Rhod5min 11367-152-33 tp4 MeOH Decon 3
MethBlue 11367-152-33 tp4 MeOH Decon 3
BF Rhod1hr 11367-152-33 tp4 MeOH Decon 2
BF Rhod5min 11367-152-33 tp4 MeOH Decon 2
BF MethBlue 11367-152-33 tp4 MeOH Decon 1
BF NoStain 11367-152-33 tp4 MeOH Decon 1
Male DC, individual 11367-152-33 at time point 4 after methanol decontamination.

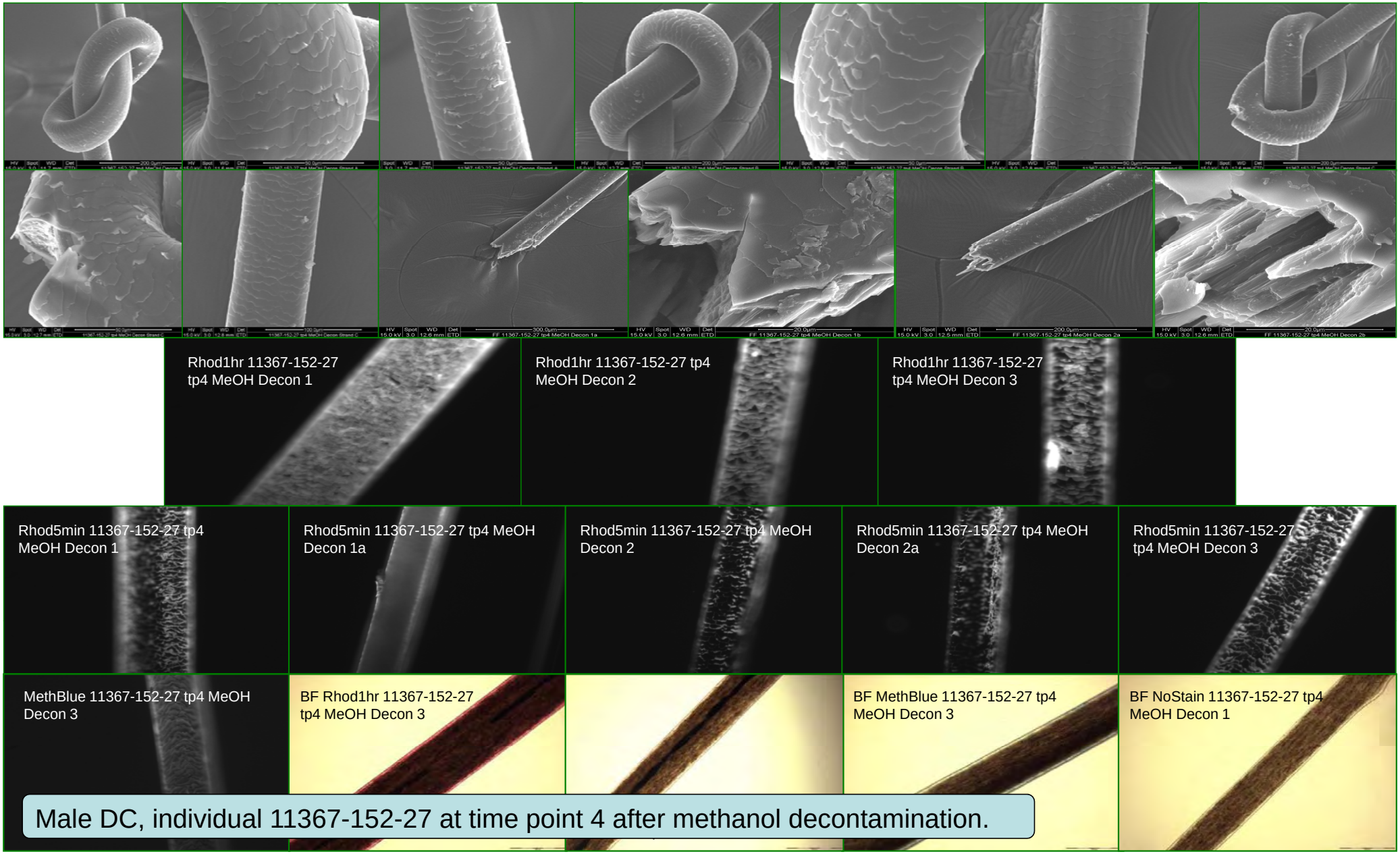

Rhod1hr 11367-152-41 tp4 MeOH Decon 1
Rhod1hr 11367-152-41 tp4 MeOH Decon 2
Rhod1hr 11367-152-41 tp4 MeOH Decon 2a
Rhod1hr 11367-152-41 tp4 MeOH Decon 3
Rhod5min 11367-152-41 tp4 MeOH Decon 1
Rhod5min 11367-152-41 tp4 MeOH Decon 2
Rhod5min 11367-152-41 tp4 MeOH Decon 2a
Rhod5min 11367-152-41 tp4 MeOH Decon 3
Rhod5min 11367-152-41 tp4 MeOH Decon 3a
MethBlue 11367-152-41 tp4 MeOH Decon 1
BF Rhod1hr 11367-152-41 tp4 MeOH Decon 1
BF Rhod5min 11367-152-41 tp4 MeOH Decon 3
BF MethBlue 11367-152-41 tp4 MeOH Decon 3
BF NoStain 11367-152-41 tp4 MeOH Decon 1
Male DC, individual 11367-152-41 at time point 4 after methanol decontamination.

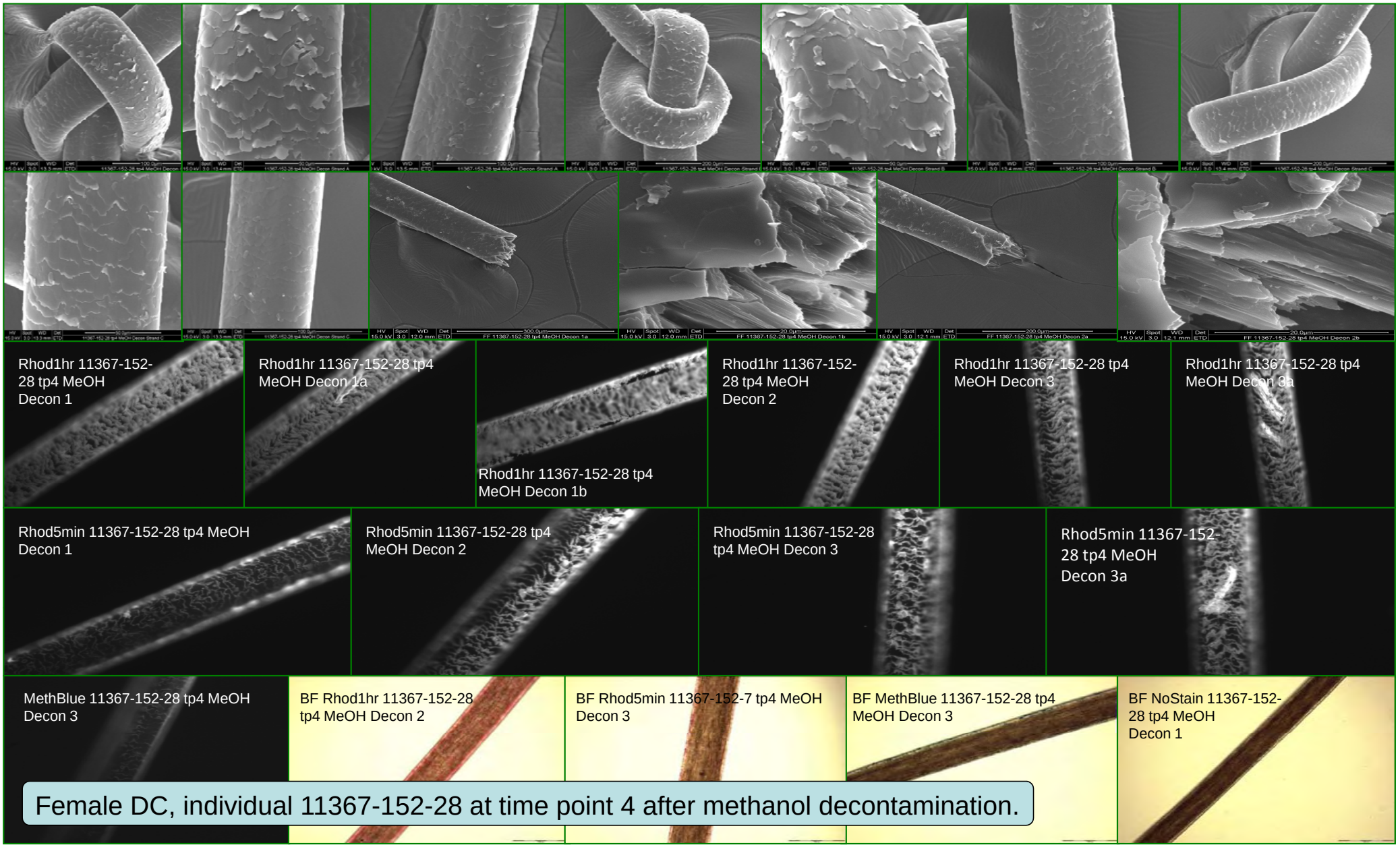


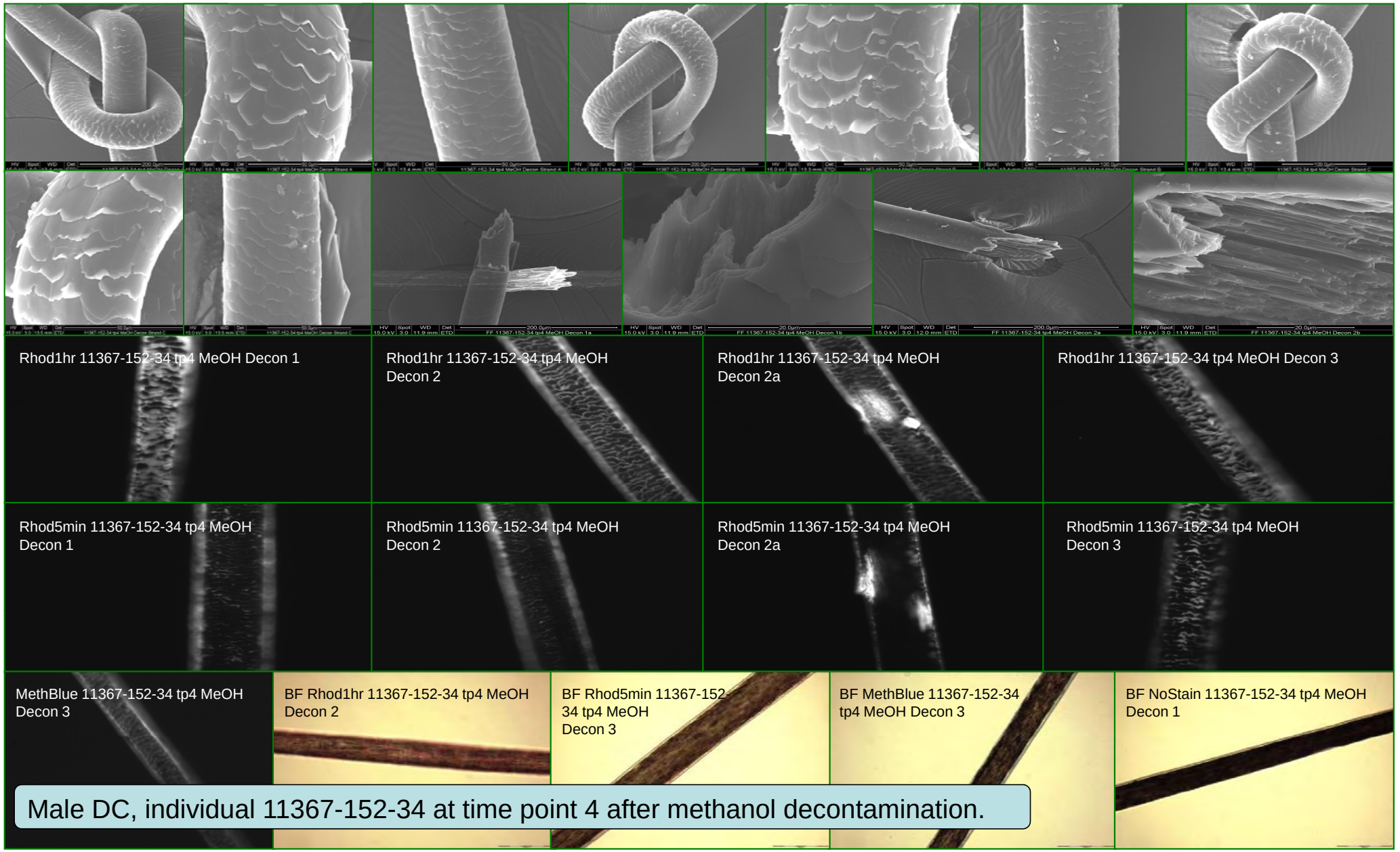


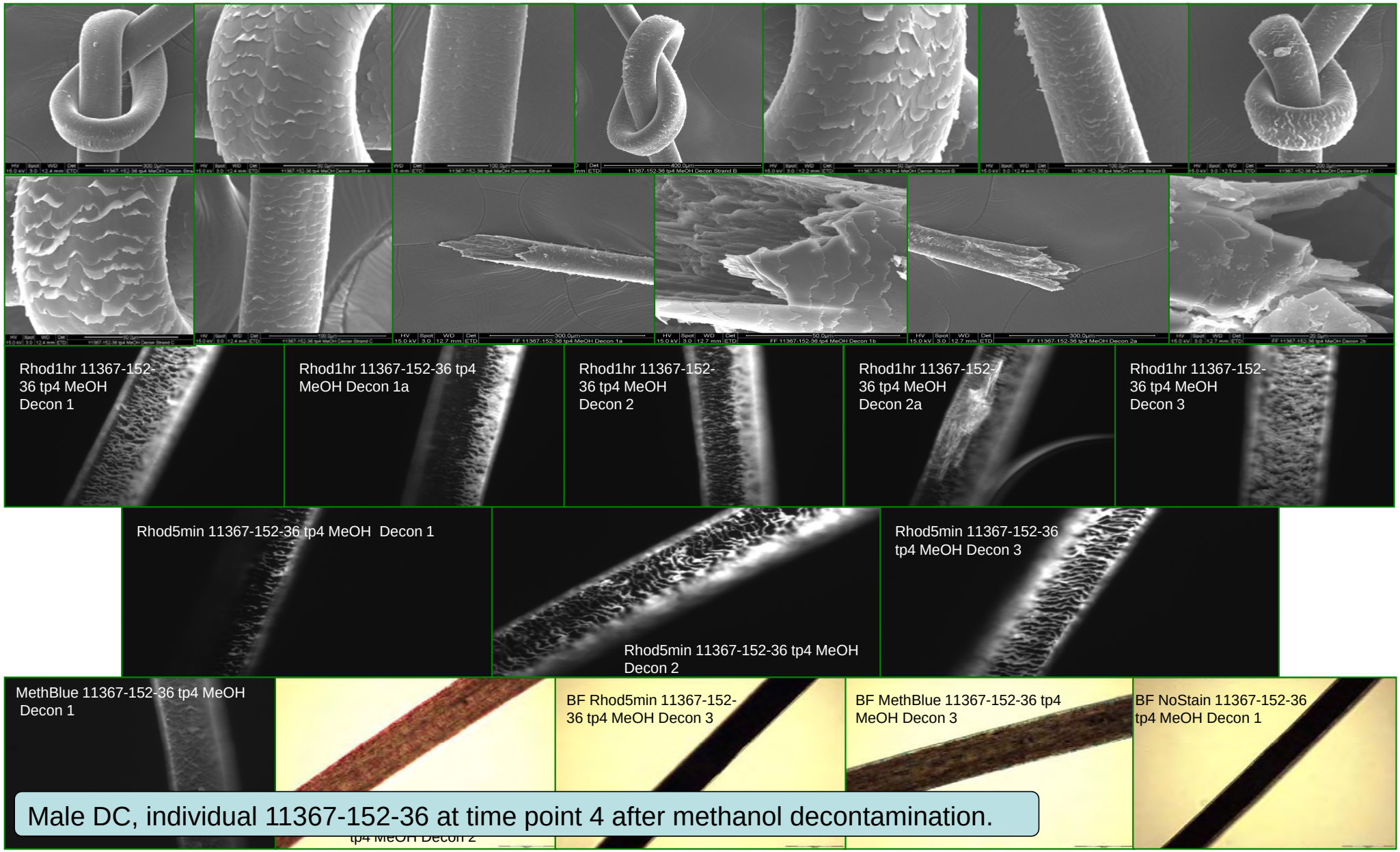


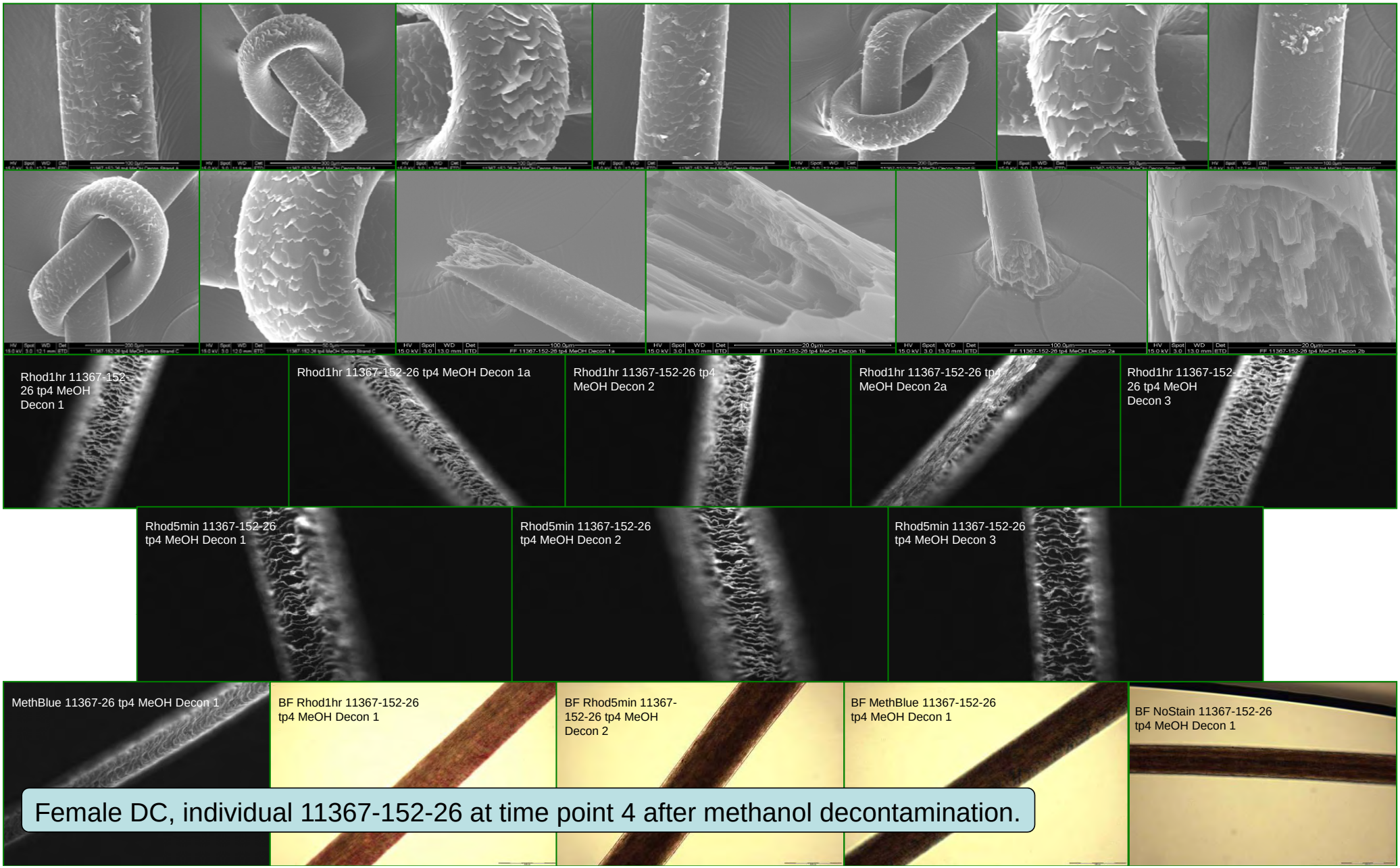
Female DC, individual 11367-152-25 at time point 4 after methanol decontamination.

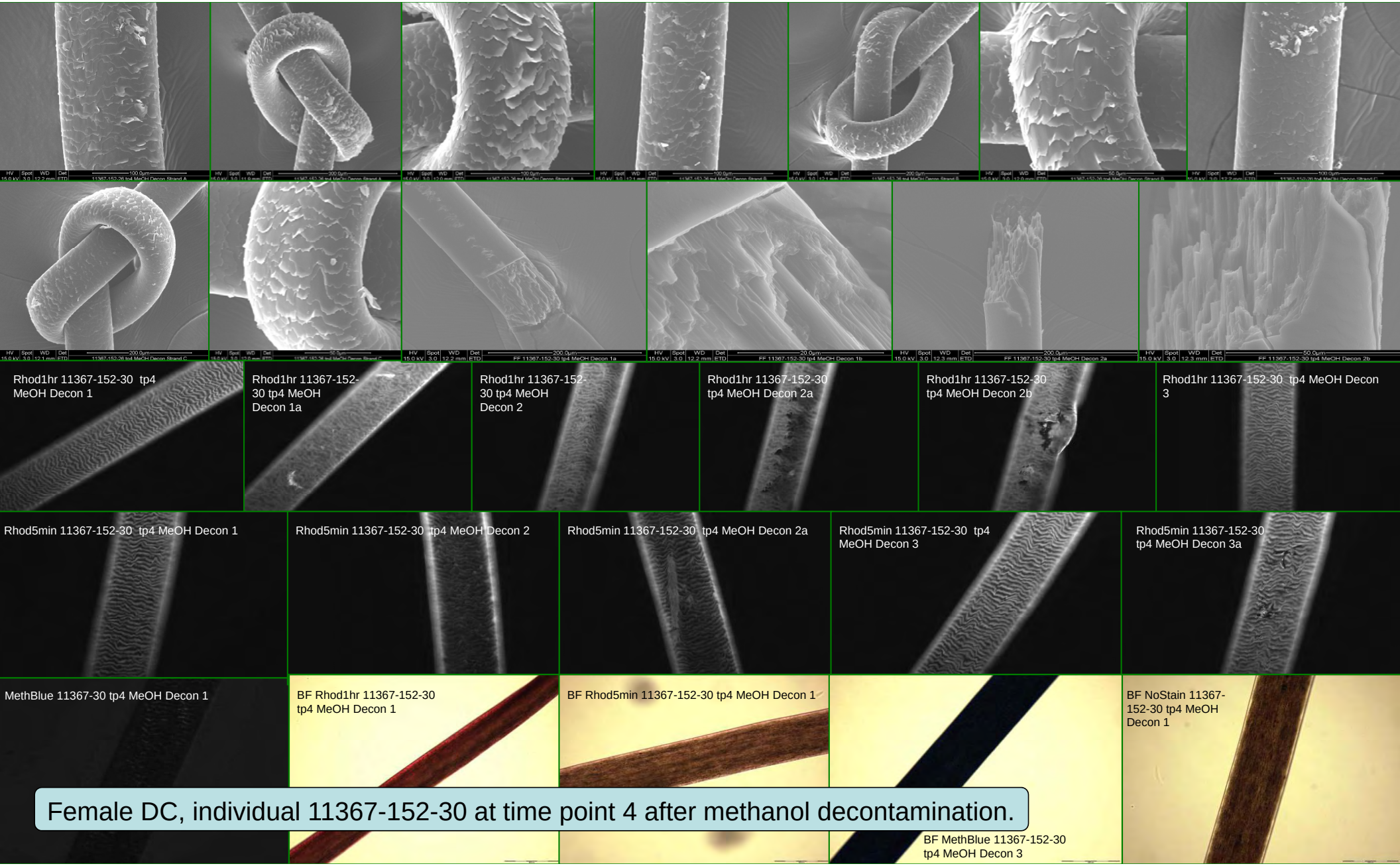

Rhod1hr 11367-152-27 tp4 MeOH Decon 1
Rhod1hr 11367-152-27 tp4 MeOH Decon 2
Rhod1hr 11367-152-27 tp4 MeOH Decon 3
Rhod5min 11367-152-27 tp4 MeOH Decon 1
Rhod5min 11367-152-27 tp4 MeOH Decon 1a
Rhod5min 11367-152-27 tp4 MeOH Decon 2
Rhod5min 11367-152-27 tp4 MeOH Decon 2a
Rhod5min 11367-152-27 tp4 MeOH Decon 3
MethBlue 11367-152-27 tp4 MeOH Decon 3
BF Rhod1hr 11367-152-27 tp4 MeOH Decon 3
BF MethBlue 11367-152-27 tp4 MeOH Decon 3
BF NoStain 11367-152-27 tp4 MeOH Decon 1
Male DC, individual 11367-152-27 at time point 4 after methanol decontamination.


Rhod1hr 11367-152-28 tp4 MeOH Decon 1
Rhod1hr 11367-152-28 tp4 MeOH Decon 1a
Rhod1hr 11367-152-28 tp4 MeOH Decon 1b
Rhod1hr 11367-152-28 tp4 MeOH Decon 2
Rhod1hr 11367-152-28 tp4 MeOH Decon 3
Rhod1hr 11367-152-28 tp4 MeOH Decon 3a
Rhod5min 11367-152-28 tp4 MeOH Decon 1
Rhod5min 11367-152-28 tp4 MeOH Decon 2
Rhod5min 11367-152-28 tp4 MeOH Decon 3
Rhod5min 11367-152-28 tp4 MeOH Decon 3a
MethBlue 11367-152-28 tp4 MeOH Decon 3
BF Rhod1hr 11367-152-28 tp4 MeOH Decon 2
BF Rhod5min 11367-152-7 tp4 MeOH Decon 3
BF MethBlue 11367-152-28 tp4 MeOH Decon 3
BF NoStain 11367-152-28 tp4 MeOH Decon 1
Female DC, individual 11367-152-28 at time point 4 after methanol decontamination.

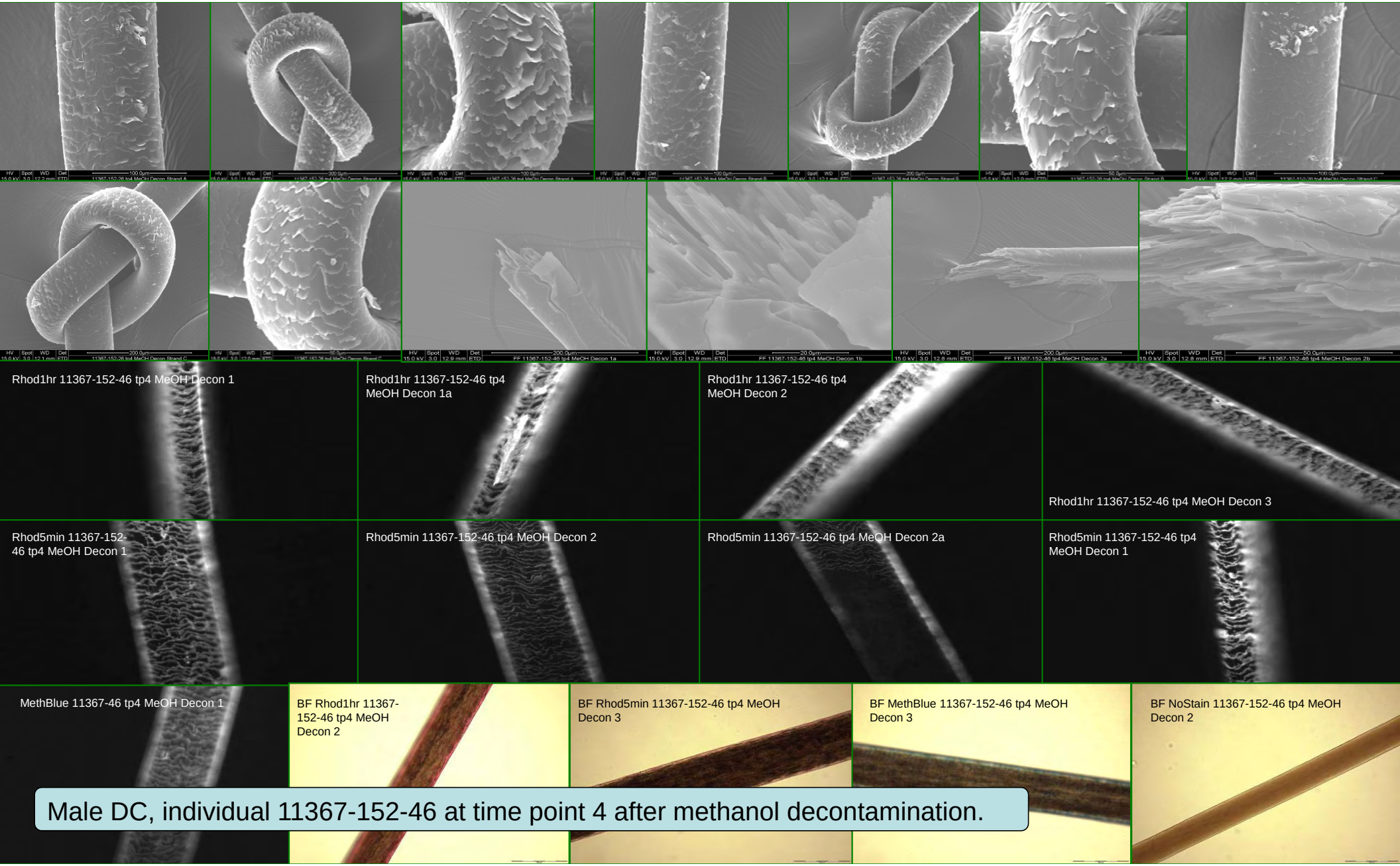

Rhod1hr 11367-152-34 tp4 MeOH Decon 1
Rhod1hr 11367-152-34 tp4 MeOH Decon 2
Rhod1hr 11367-152-34 tp4 MeOH Decon 2a
Rhod1hr 11367-152-34 tp4 MeOH Decon 3
Rhod5min 11367-152-34 tp4 MeOH Decon 1
Rhod5min 11367-152-34 tp4 MeOH Decon 2
Rhod5min 11367-152-34 tp4 MeOH Decon 2a
Rhod5min 11367-152-34 tp4 MeOH Decon 3
MethBlue 11367-152-34 tp4 MeOH Decon 3
BF Rhod1hr 11367-152-34 tp4 MeOH Decon 2
BF Rhod5min 11367-152-34 tp4 MeOH Decon 3
BF MethBlue 11367-152-34 tp4 MeOH Decon 3
BF NoStain 11367-152-34 tp4 MeOH Decon 1
Male DC, individual 11367-152-34 at time point 4 after methanol decontamination.


Rhod1hr 11367-152-36 tp4 MeOH Decon 1
Rhod1hr 11367-152-36 tp4 MeOH Decon 1a
Rhod1hr 11367-152-36 tp4 MeOH Decon 2
Rhod1hr 11367-152-36 tp4 MeOH Decon 2a
Rhod1hr 11367-152-36 tp4 MeOH Decon 3
Rhod5min 11367-152-36 tp4 MeOH Decon 1
Rhod5min 11367-152-36 tp4 MeOH Decon 2
Rhod5min 11367-152-36 tp4 MeOH Decon 3
MethBlue 11367-152-36 tp4 MeOH Decon 1
BF Rhod5min 11367-152-36 tp4 MeOH Decon 3
BF MethBlue 11367-152-36 tp4 MeOH Decon 3
BF NoStain 11367-152-36 tp4 MeOH Decon 1
Male DC, individual 11367-152-36 at time point 4 after methanol decontamination.


Rhod1hr 11367-152-26 tp4 MeOH Decon 1
Rhod1hr 11367-152-26 tp4 MeOH Decon 1a
Rhod1hr 11367-152-26 tp4 MeOH Decon 2
Rhod1hr 11367-152-26 tp4 MeOH Decon 2a
Rhod1hr 11367-152-26 tp4 MeOH Decon 3
Rhod5min 11367-152-26 tp4 MeOH Decon 1
Rhod5min 11367-152-26 tp4 MeOH Decon 2
Rhod5min 11367-152-26 tp4 MeOH Decon 3
MethBlue 11367-26 tp4 MeOH Decon 1
BF Rhod1hr 11367-152-26 tp4 MeOH Decon 1
BF Rhod5min 11367-152-26 tp4 MeOH Decon 2
BF MethBlue 11367-152-26 tp4 MeOH Decon 1
BF NoStain 11367-152-26 tp4 MeOH Decon 1
Female DC, individual 11367-152-26 at time point 4 after methanol decontamination.



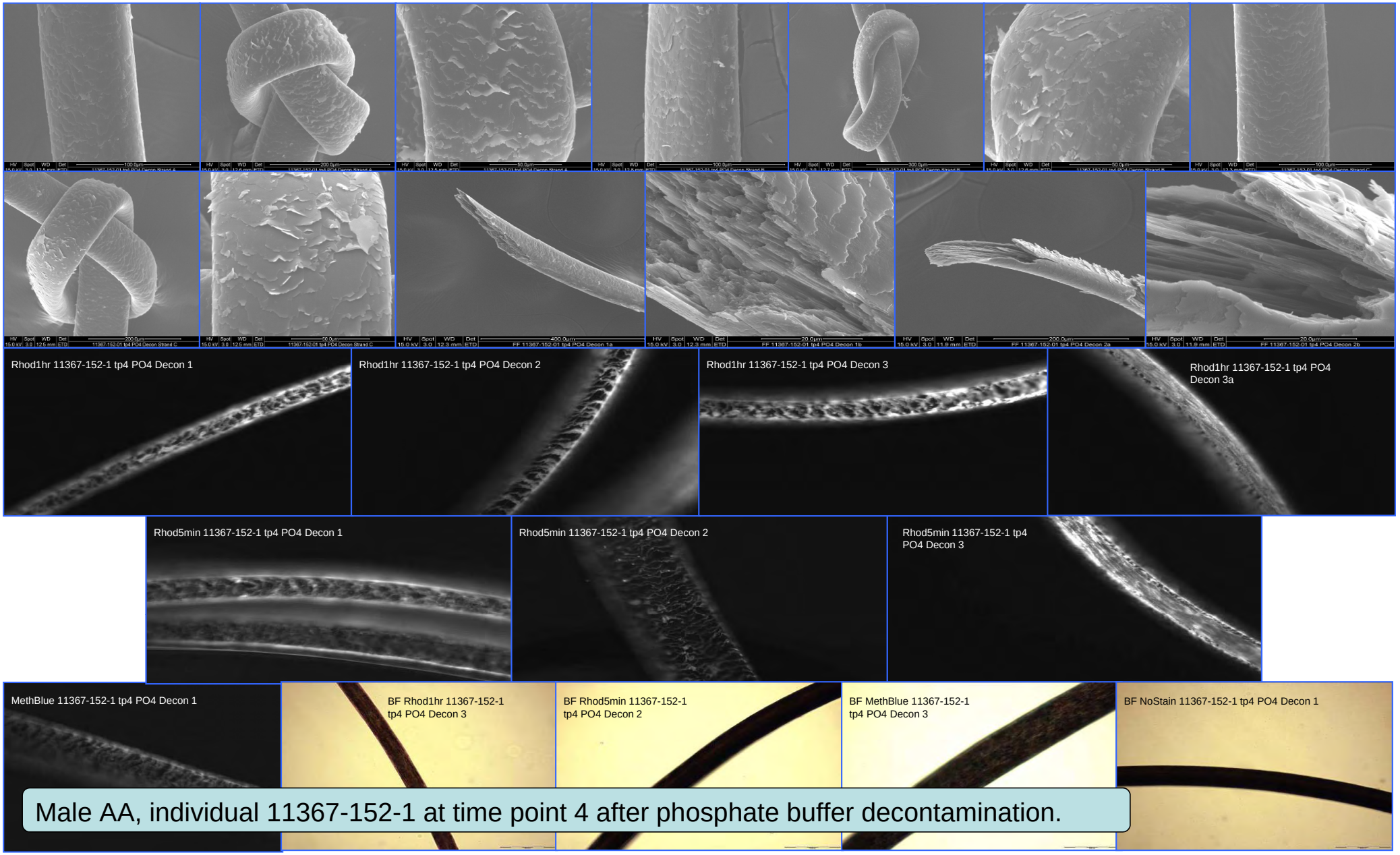
Female DC, individual 11367-152-30 at time point 4 after methanol decontamination.



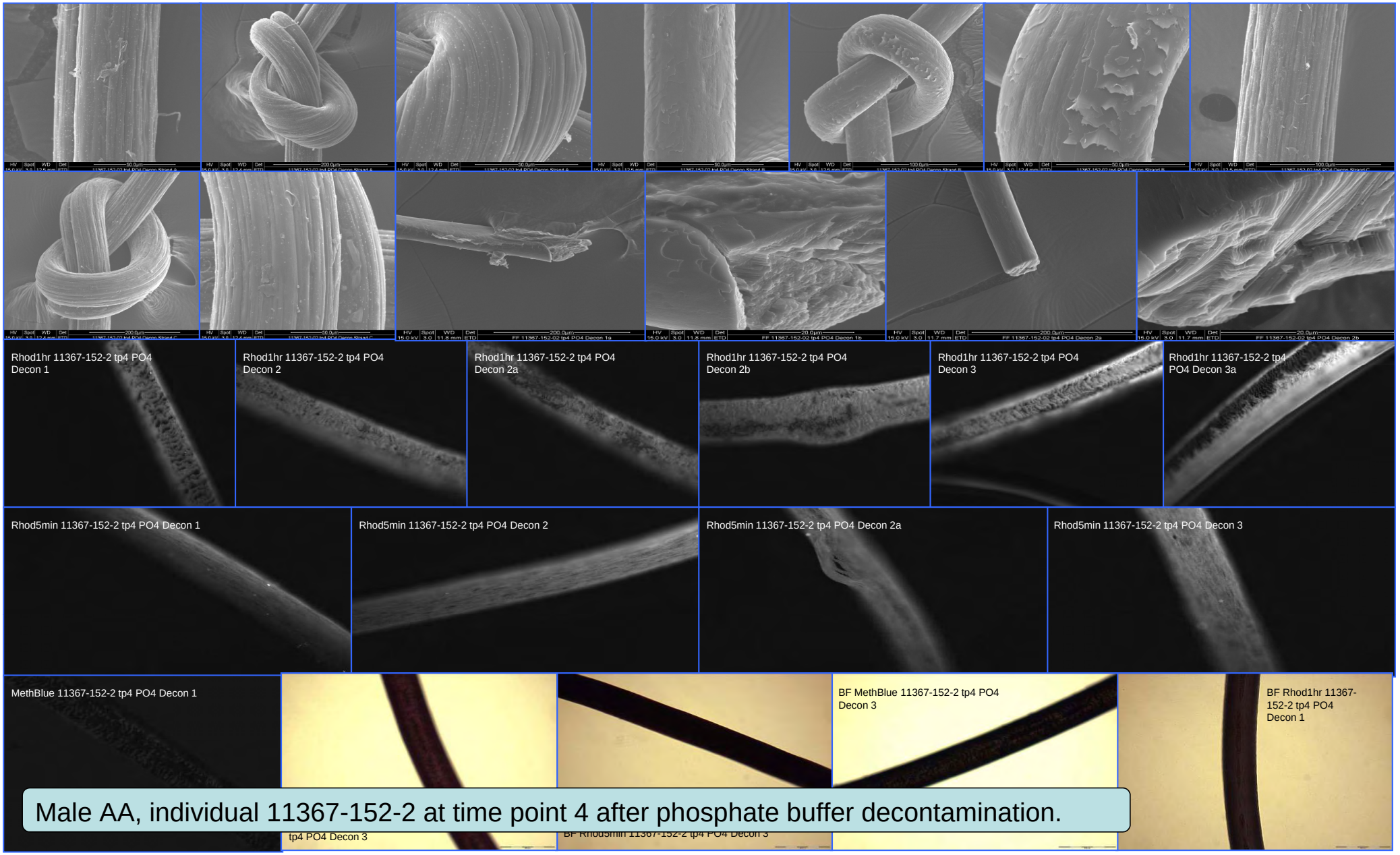
Male DC, individual 11367-152-46 at time point 4 after methanol decontamination.

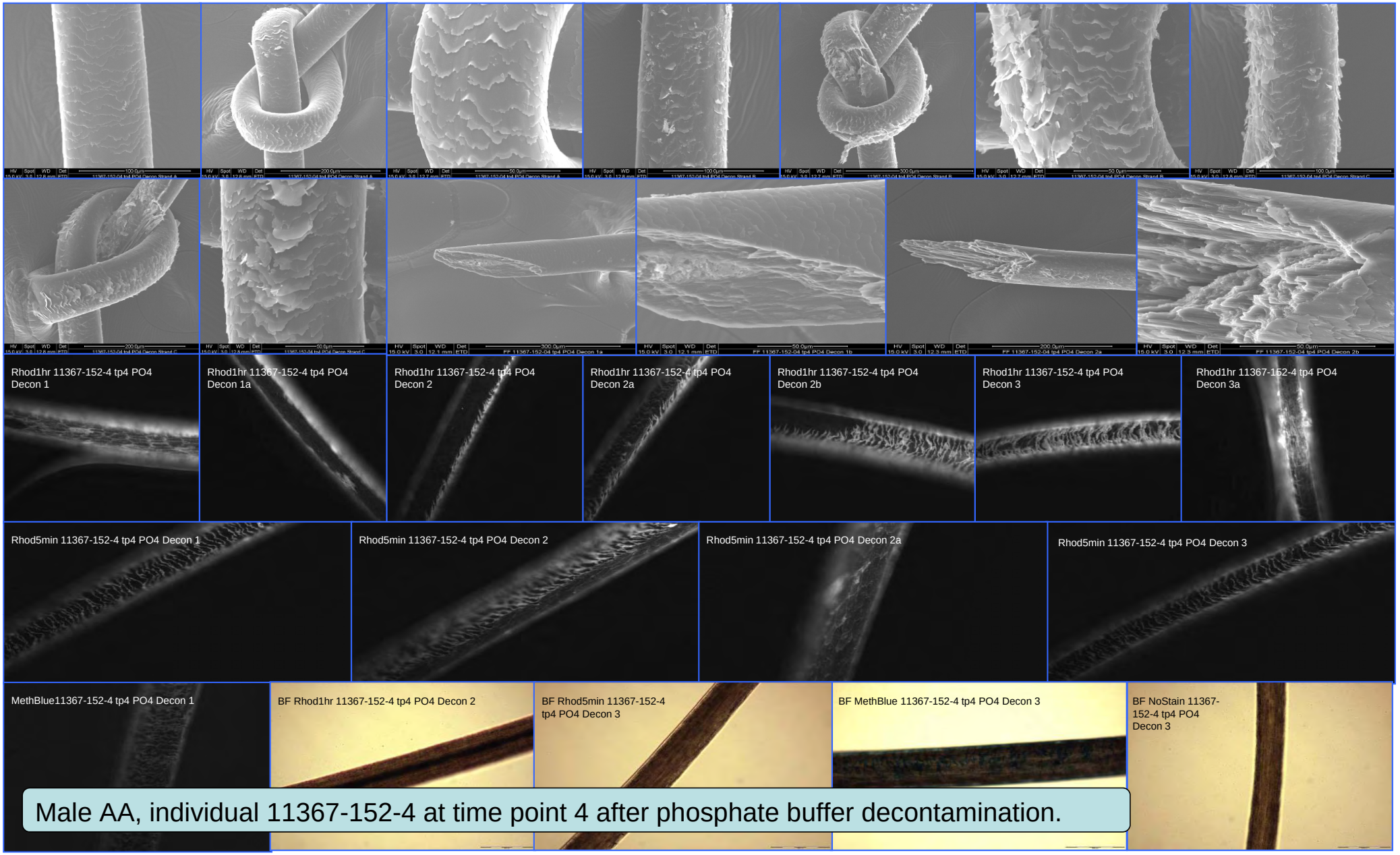
Appendix C, Part 3

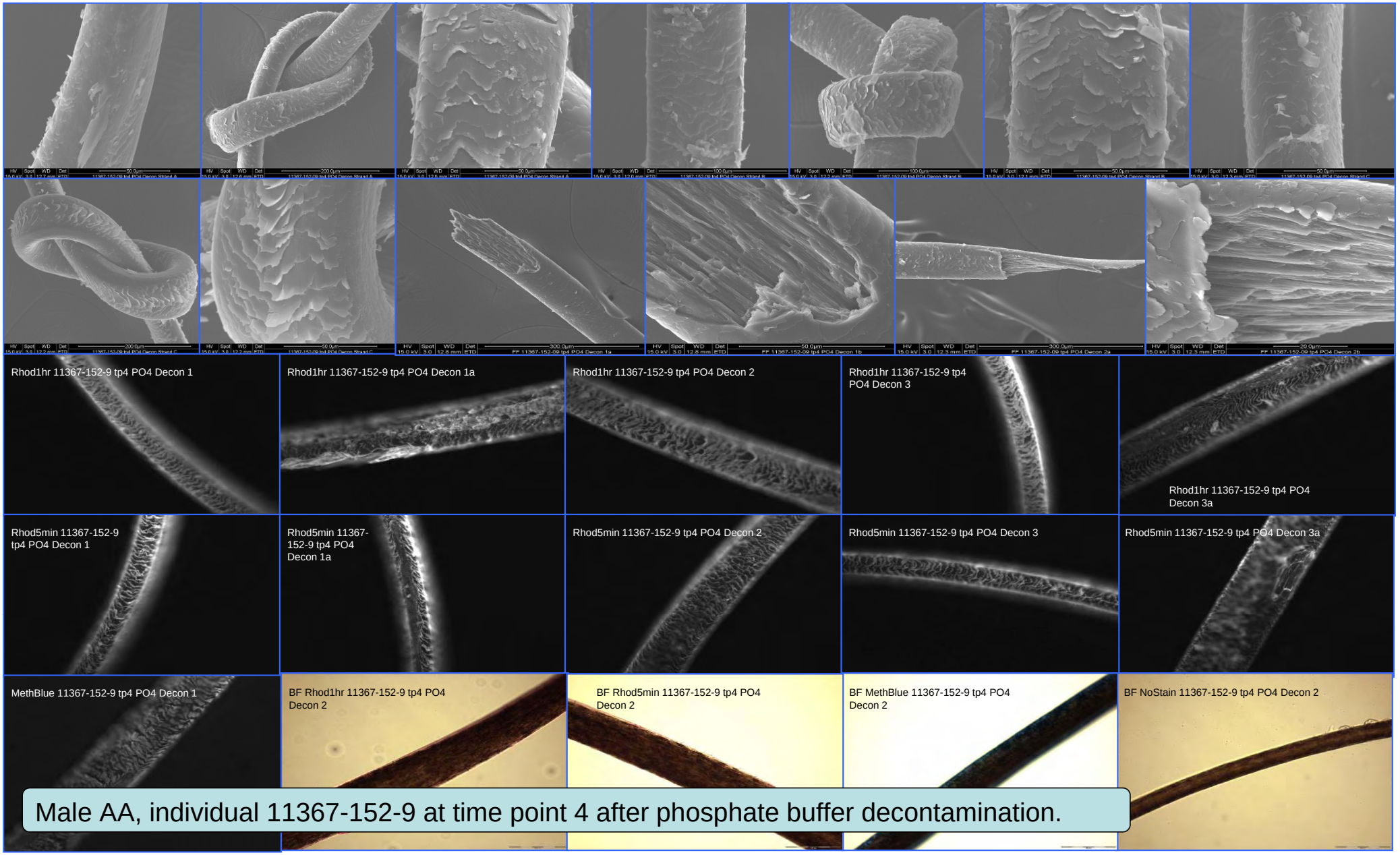
Individuals at Time Point 4 After Phosphate Buffer Decontamination



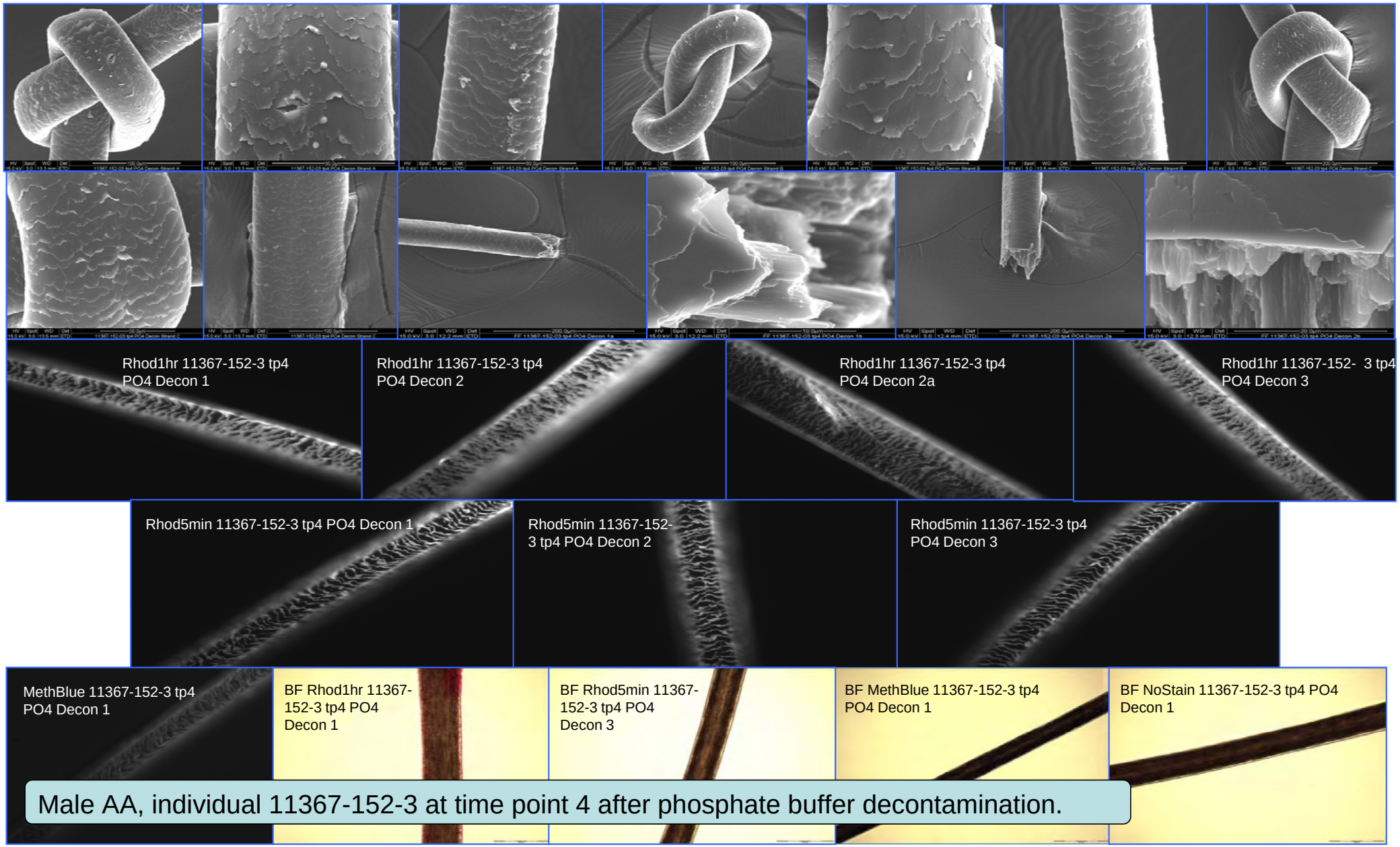
Male AA, individual 11367-152-1 at time point 4 after phosphate buffer decontamination.

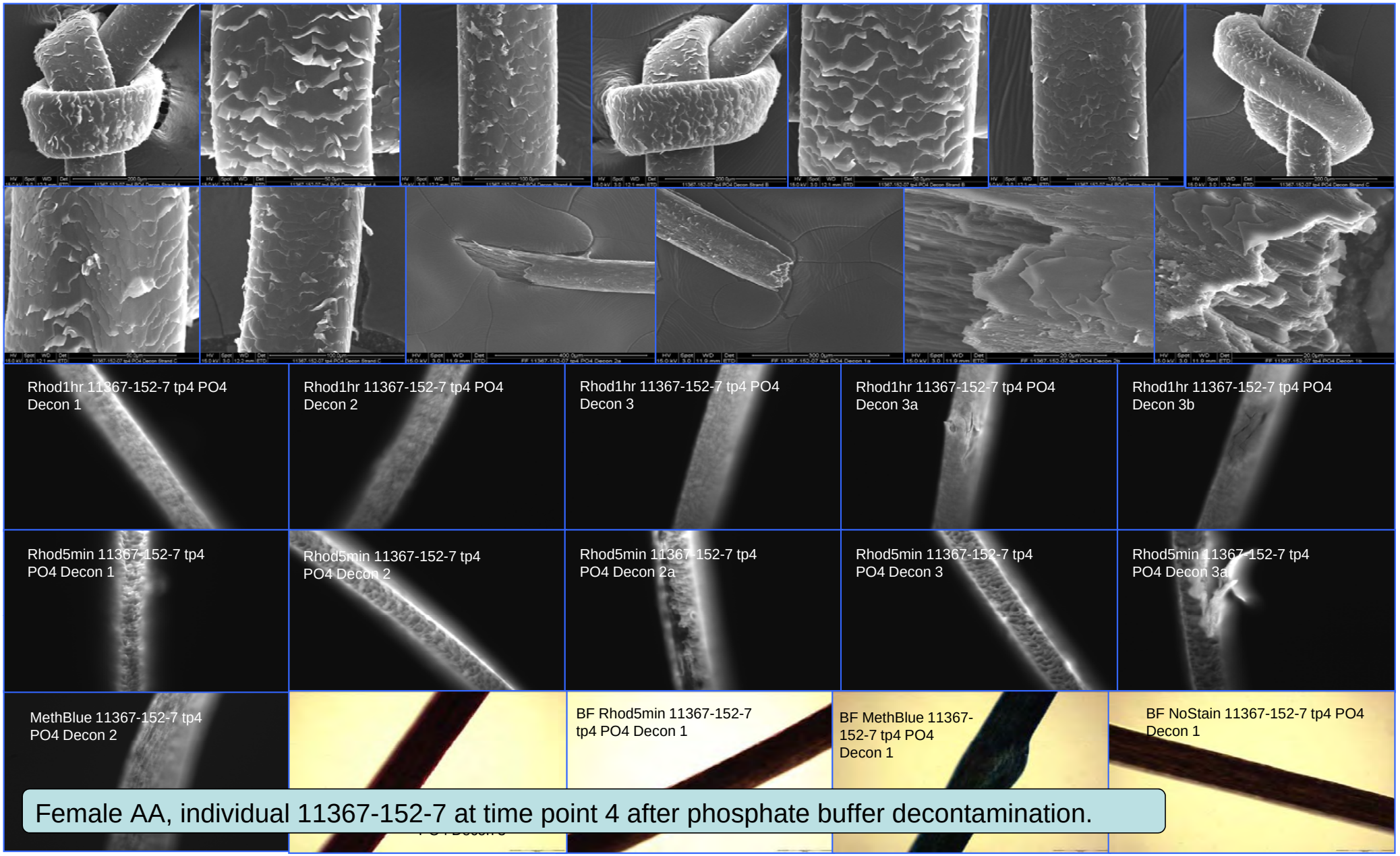

Rhod1hr 11367-152-2 tp4 PO4 Decon 1
Rhod1hr 11367-152-2 tp4 PO4 Decon 2
Rhod1hr 11367-152-2 tp4 PO4 Decon 2a
Rhod1hr 11367-152-2 tp4 PO4 Decon 2b
Rhod1hr 11367-152-2 tp4 PO4 Decon 3
Rhod1hr 11367-152-2 tp4 PO4 Decon 3a
Rhod5min 11367-152-2 tp4 PO4 Decon 1
Rhod5min 11367-152-2 tp4 PO4 Decon 2
Rhod5min 11367-152-2 tp4 PO4 Decon 2a
Rhod5min 11367-152-2 tp4 PO4 Decon 3
MethBlue 11367-152-2 tp4 PO4 Decon 1
BF MethBlue 11367-152-2 tp4 PO4 Decon 3
BF Rhod1hr 11367-152-2 tp4 PO4 Decon 1
Male AA, individual 11367-152-2 at time point 4 after phosphate buffer decontamination.

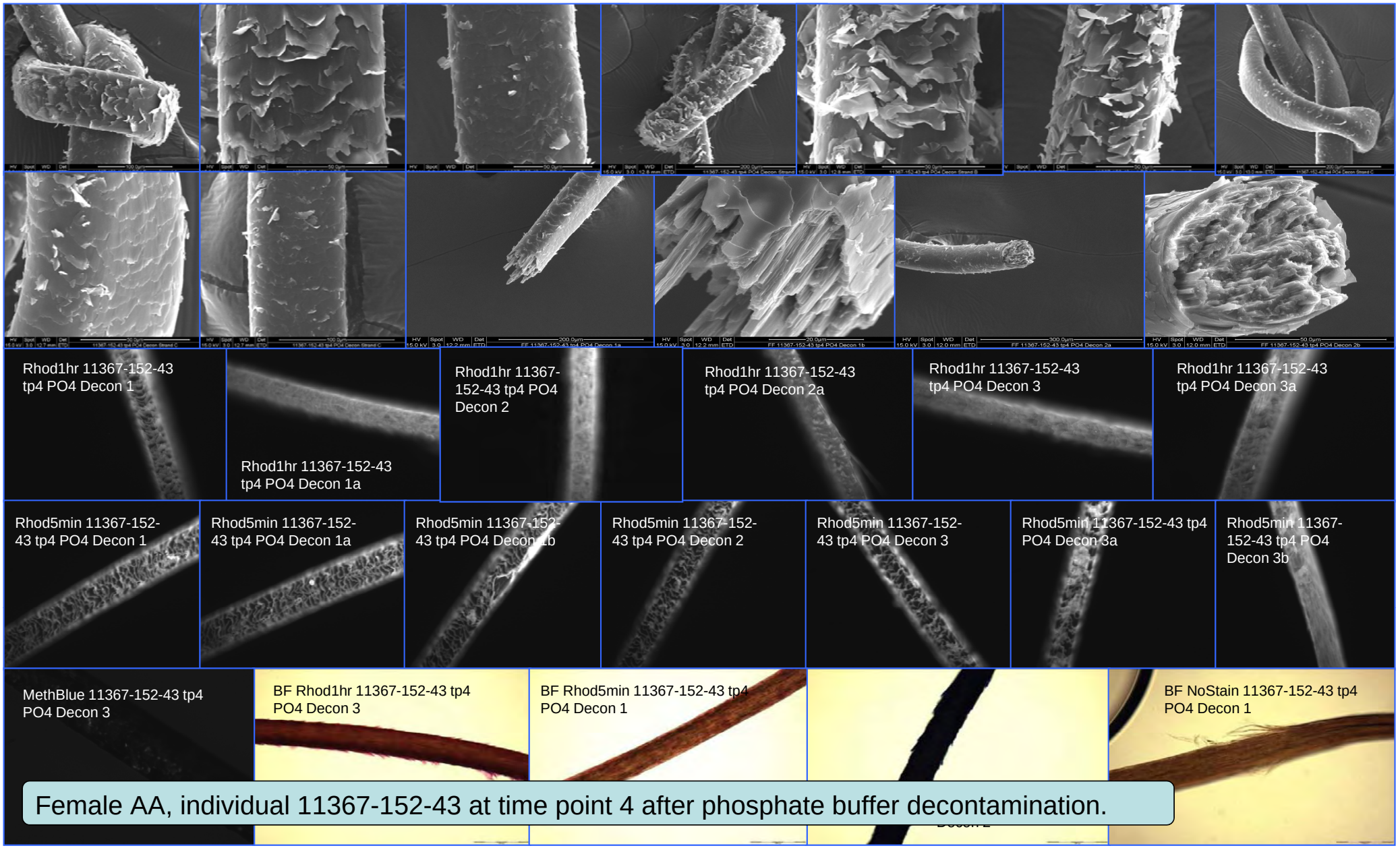

Rhod1hr 11367-152-4 tp4 PO4 Decon 1
Rhod1hr 11367-152-4 tp4 PO4 Decon 1a
Rhod1hr 11367-152-4 tp4 PO4 Decon 2
Rhod1hr 11367-152-4 tp4 PO4 Decon 2a
Rhod1hr 11367-152-4 tp4 PO4 Decon 2b
Rhod1hr 11367-152-4 tp4 PO4 Decon 3
Rhod1hr 11367-152-4 tp4 PO4 Decon 3a
Rhod5min 11367-152-4 tp4 PO4 Decon 1
Rhod5min 11367-152-4 tp4 PO4 Decon 2
Rhod5min 11367-152-4 tp4 PO4 Decon 2a
Rhod5min 11367-152-4 tp4 PO4 Decon 3
MethBlue11367-152-4 tp4 PO4 Decon 1
BF Rhod1hr 11367-152-4 tp4 PO4 Decon 2
BF Rhod5min 11367-152-4 tp4 PO4 Decon 3
BF MethBlue 11367-152-4 tp4 PO4 Decon 3
BF NoStain 11367-152-4 tp4 PO4 Decon 3
Male AA, individual 11367-152-4 at time point 4 after phosphate buffer decontamination.

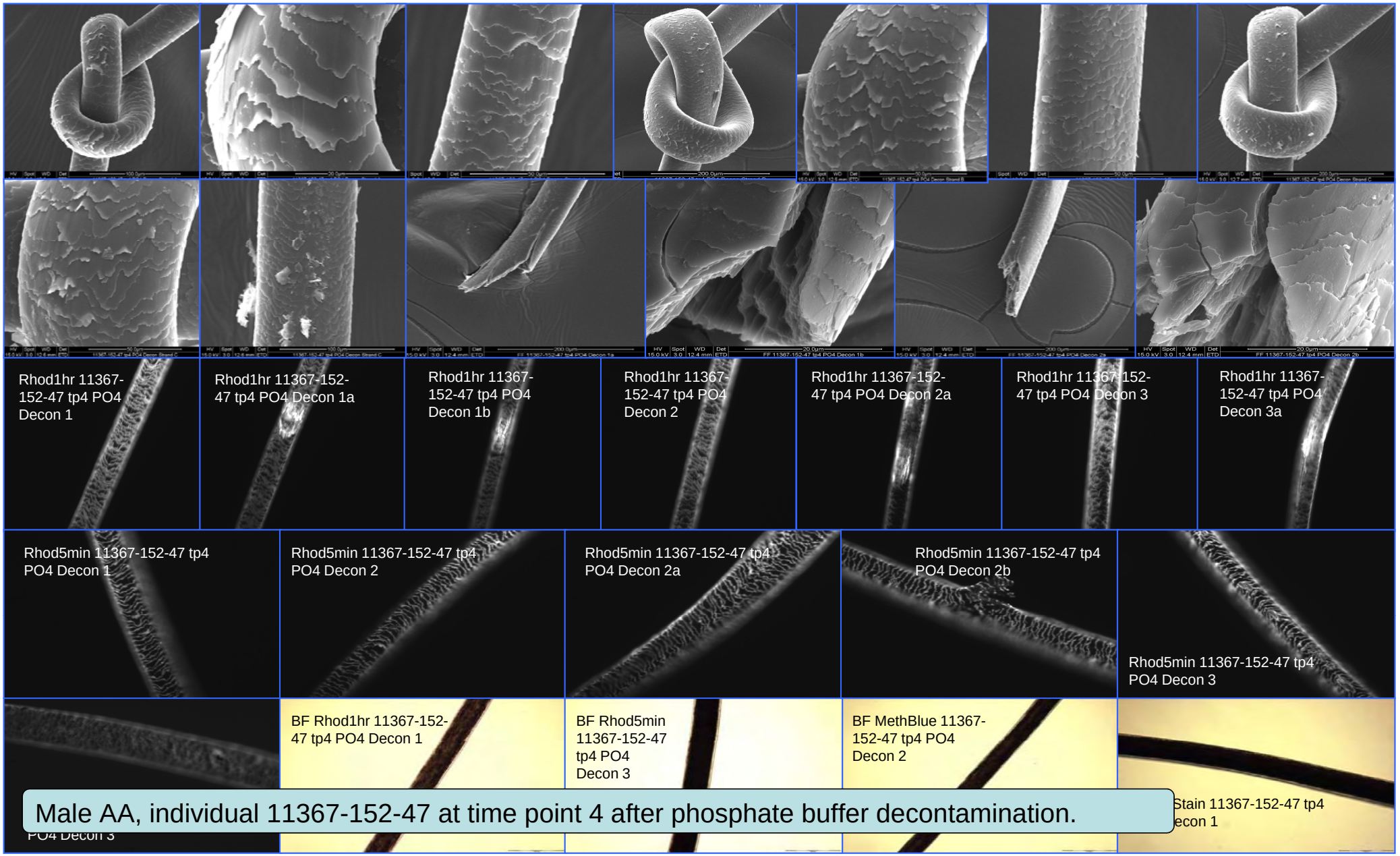


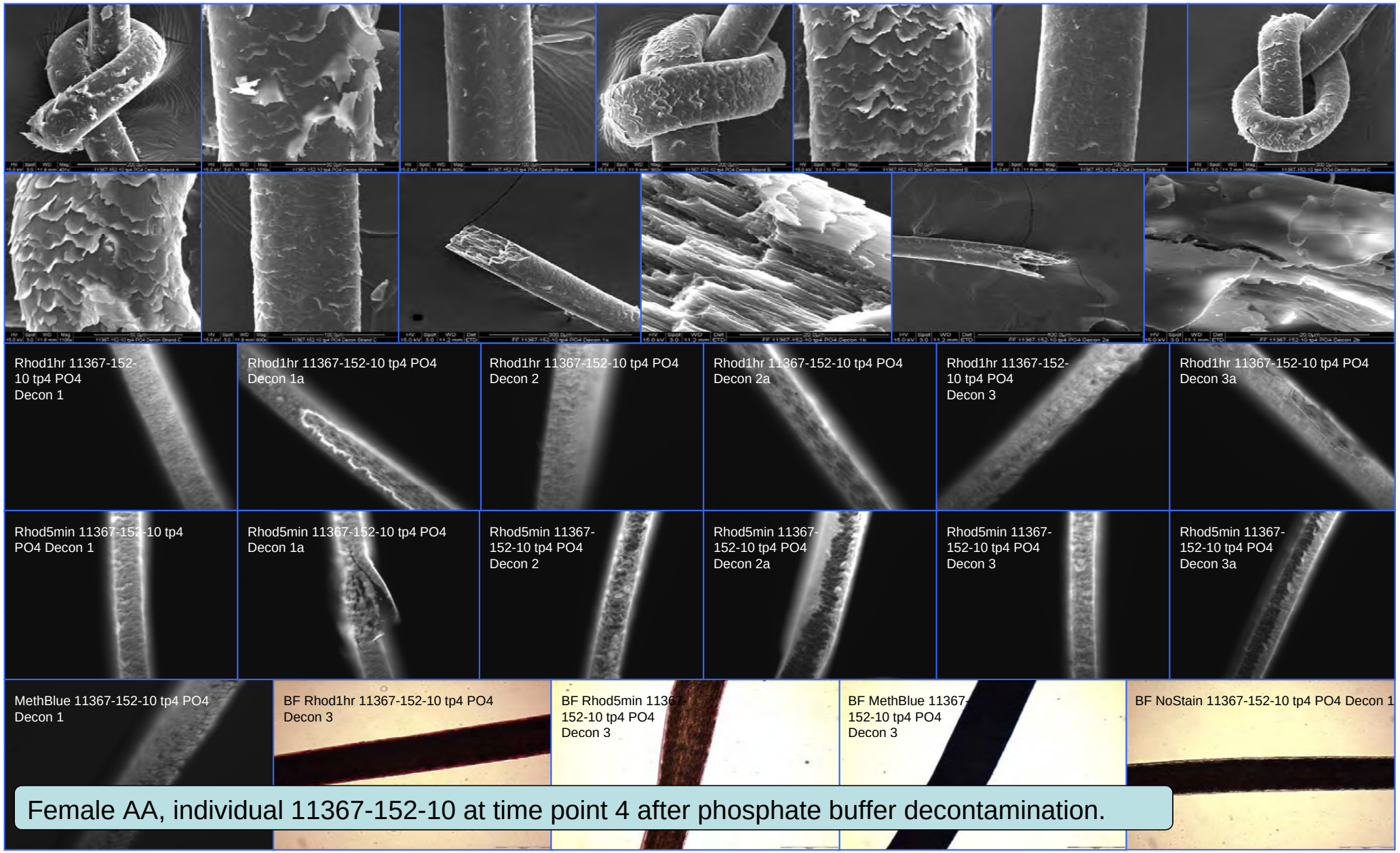
Male AA, individual 11367-152-9 at time point 4 after phosphate buffer decontamination.

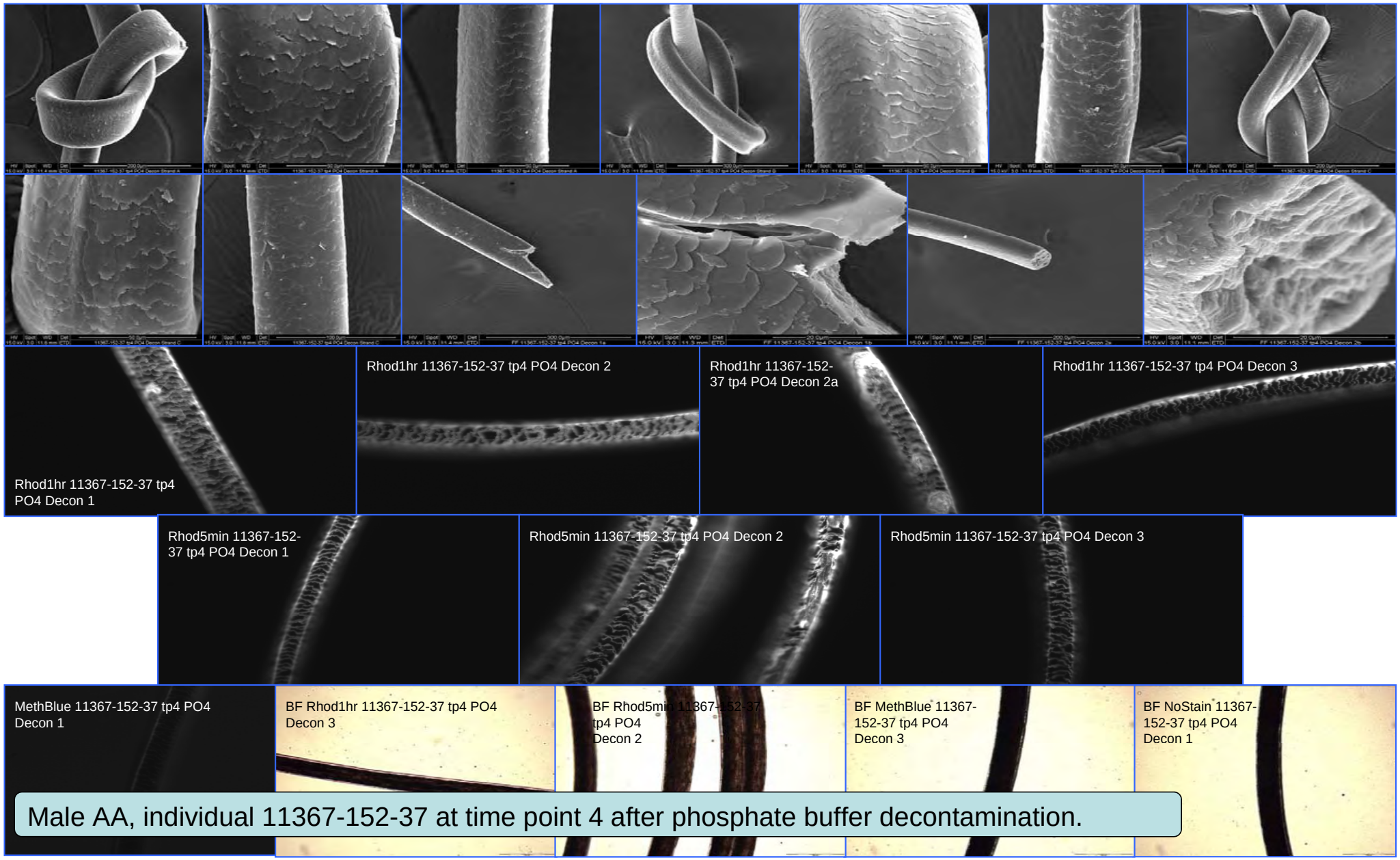

Rhod1hr 11367-152-3 tp4 PO4 Decon 1
Rhod1hr 11367-152-3 tp4 PO4 Decon 2
Rhod1hr 11367-152-3 tp4 PO4 Decon 2a
Rhod1hr 11367-152- 3 tp4 PO4 Decon 3
Rhod5min 11367-152-3 tp4 PO4 Decon 1
Rhod5min 11367-152-3 tp4 PO4 Decon 2
Rhod5min 11367-152-3 tp4 PO4 Decon 3
MethBlue 11367-152-3 tp4 PO4 Decon 1
BF Rhod1hr 11367-152-3 tp4 PO4 Decon 1
BF Rhod5min 11367-152-3 tp4 PO4 Decon 3
BF MethBlue 11367-152-3 tp4 PO4 Decon 1
BF NoStain 11367-152-3 tp4 PO4 Decon 1
Male AA, individual 11367-152-3 at time point 4 after phosphate buffer decontamination.


Rhod1hr 11367-152-7 tp4 PO4 Decon 1
Rhod1hr 11367-152-7 tp4 PO4 Decon 2
Rhod1hr 11367-152-7 tp4 PO4 Decon 3
Rhod1hr 11367-152-7 tp4 PO4 Decon 3a
Rhod1hr 11367-152-7 tp4 PO4 Decon 3b
Rhod5min 11367-152-7 tp4 PO4 Decon 1
Rhod5min 11367-152-7 tp4 PO4 Decon 2
Rhod5min 11367-152-7 tp4 PO4 Decon 2a
Rhod5min 11367-152-7 tp4 PO4 Decon 3
Rhod5min 11367-152-7 tp4 PO4 Decon 3a
MethBlue 11367-152-7 tp4 PO4 Decon 2
BF Rhod5min 11367-152-7 tp4 PO4 Decon 1
BF MethBlue 11367-152-7 tp4 PO4 Decon 1
BF NoStain 11367-152-7 tp4 PO4 Decon 1
Female AA, individual 11367-152-7 at time point 4 after phosphate buffer decontamination.

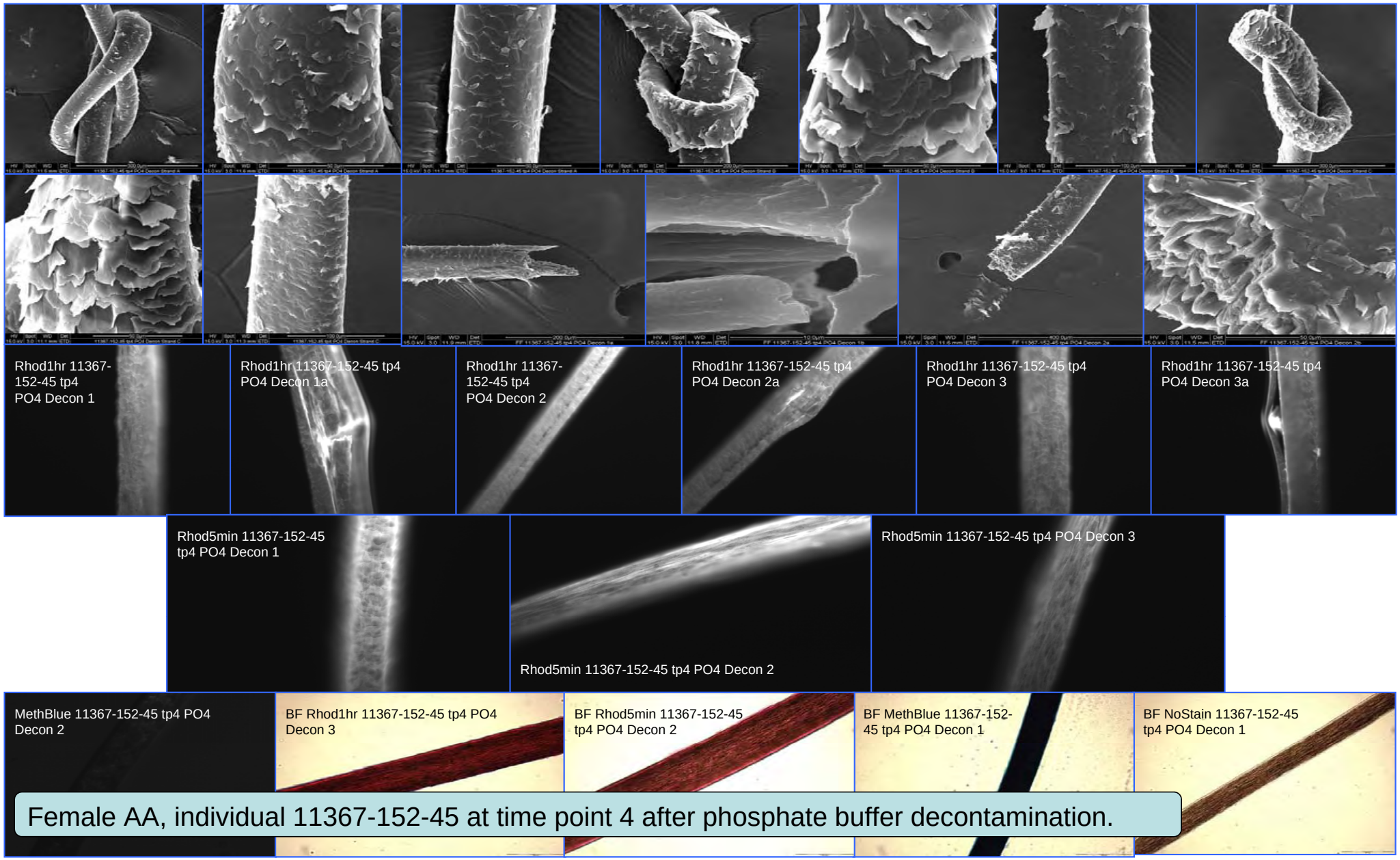

Rhod1hr 11367-152-43 tp4 PO4 Decon 1
Rhod1hr 11367-152-43 tp4 PO4 Decon 1a
Rhod1hr 11367-152-43 tp4 PO4 Decon 2
Rhod1hr 11367-152-43 tp4 PO4 Decon 2a
Rhod1hr 11367-152-43 tp4 PO4 Decon 3
Rhod1hr 11367-152-43 tp4 PO4 Decon 3a
Rhod5min 11367-152-43 tp4 PO4 Decon 1
Rhod5min 11367-152-43 tp4 PO4 Decon 1a
Rhod5min 11367-152-43 tp4 PO4 Decon 1b
Rhod5min 11367-152-43 tp4 PO4 Decon 2
Rhod5min 11367-152-43 tp4 PO4 Decon 3
Rhod5min 11367-152-43 tp4 PO4 Decon 3a
Rhod5min 11367-152-43 tp4 PO4 Decon 3b
MethBlue 11367-152-43 tp4 PO4 Decon 3
BF Rhod1hr 11367-152-43 tp4 PO4 Decon 3
BF Rhod5min 11367-152-43 tp4 PO4 Decon 1
BF NoStain 11367-152-43 tp4 PO4 Decon 1
Female AA, individual 11367-152-43 at time point 4 after phosphate buffer decontamination.

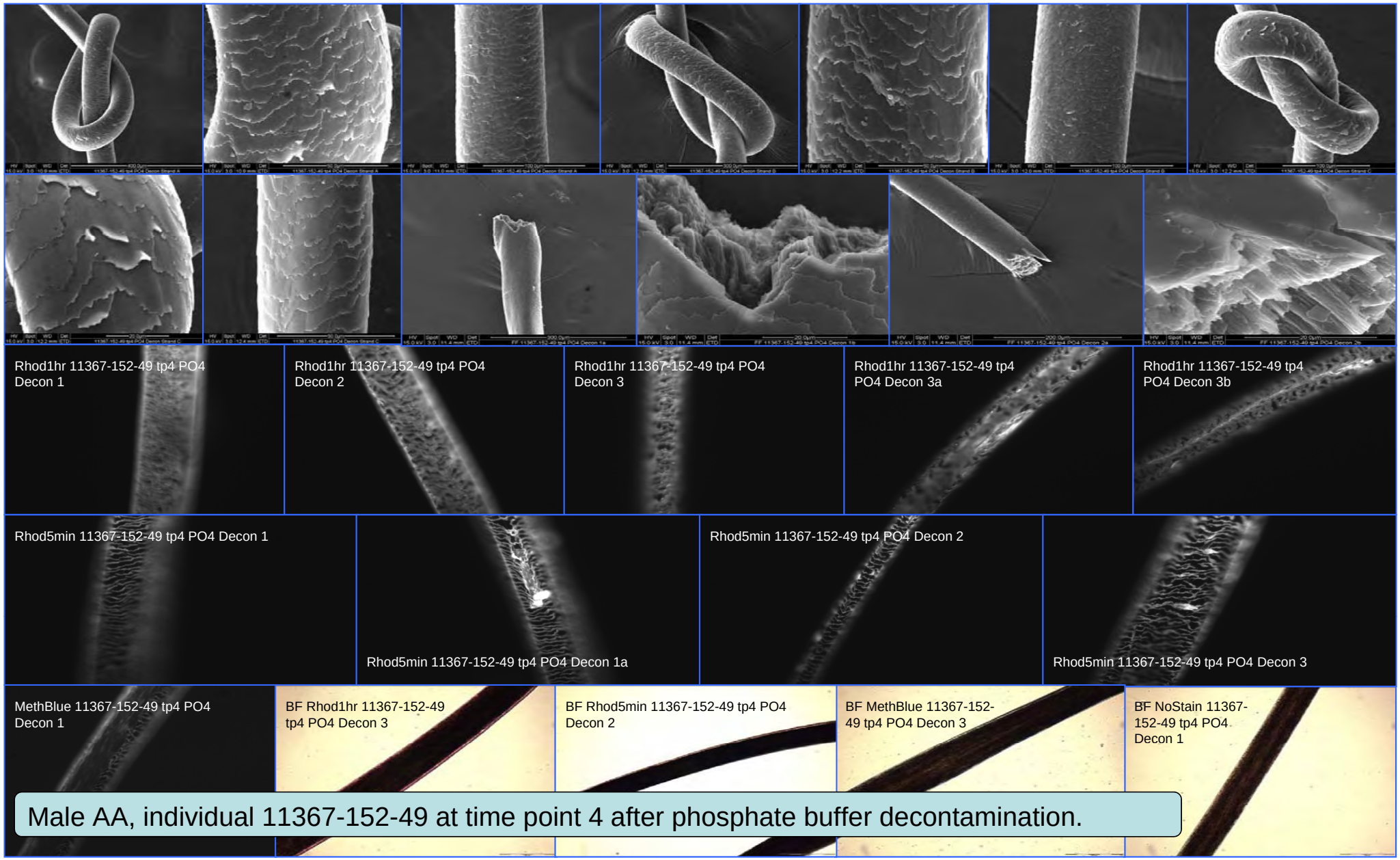

Rhod1hr 11367-152-47 tp4 PO4 Decon 1
Rhod1hr 11367-152-47 tp4 PO4 Decon 1a
Rhod1hr 11367-152-47 tp4 PO4 Decon 1b
Rhod1hr 11367-152-47 tp4 PO4 Decon 2
Rhod1hr 11367-152-47 tp4 PO4 Decon 2a
Rhod1hr 11367-152-47 tp4 PO4 Decon 3
Rhod1hr 11367-152-47 tp4 PO4 Decon 3a
Rhod5min 11367-152-47 tp4 PO4 Decon 1
Rhod5min 11367-152-47 tp4 PO4 Decon 2
Rhod5min 11367-152-47 tp4 PO4 Decon 2a
Rhod5min 11367-152-47 tp4 PO4 Decon 2b
Rhod5min 11367-152-47 tp4 PO4 Decon 3
BF Rhod1hr 11367-152-47 tp4 PO4 Decon 1
BF Rhod5min 11367-152-47 tp4 PO4 Decon 3
BF MethBlue 11367-152-47 tp4 PO4 Decon 2
Male AA, individual 11367-152-47 at time point 4 after phosphate buffer decontamination.

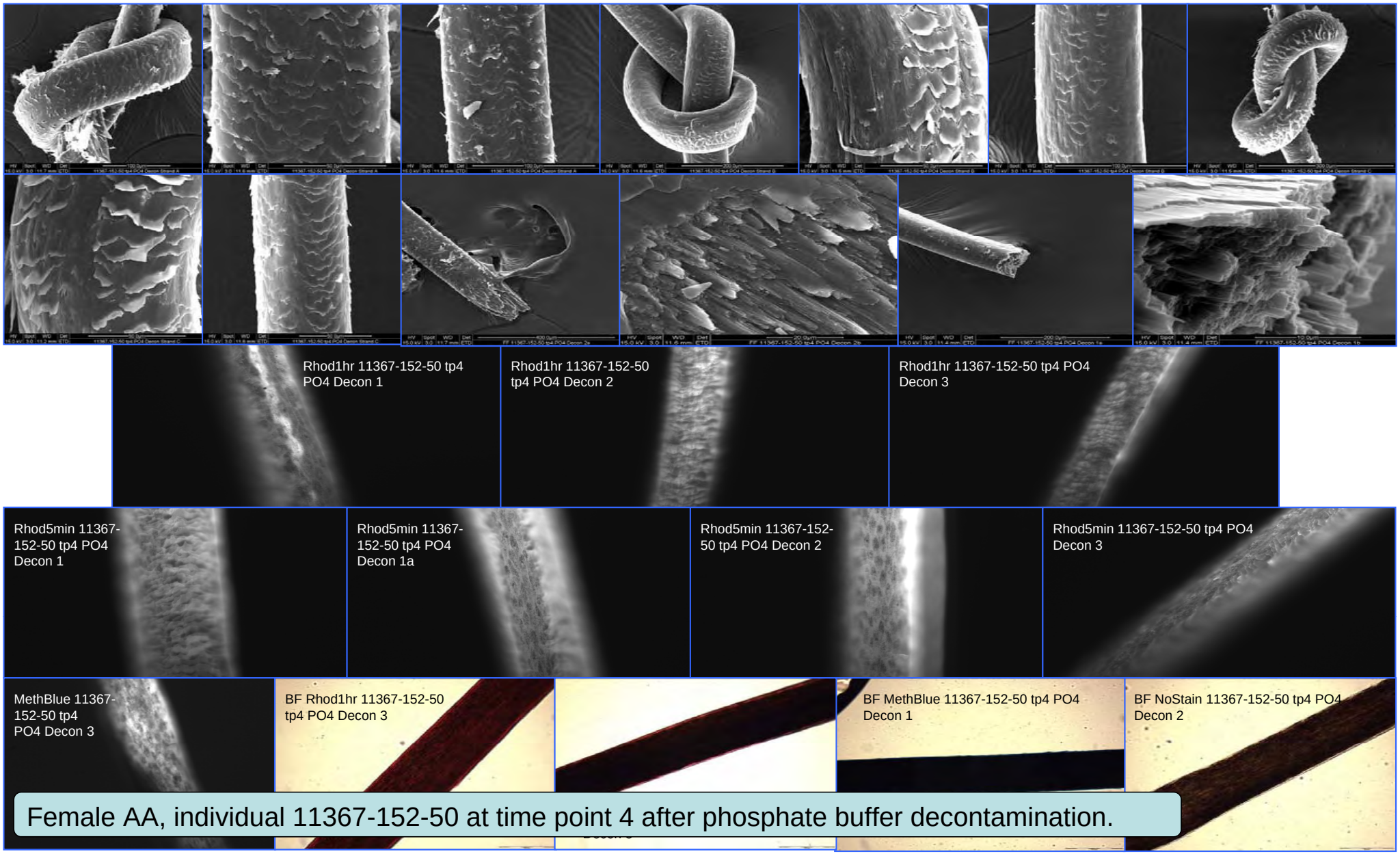

Rhod1hr 11367-152-10 tp4 PO4 Decon 1
Rhod1hr 11367-152-10 tp4 PO4 Decon 1a
Rhod1hr 11367-152-10 tp4 PO4 Decon 2
Rhod1hr 11367-152-10 tp4 PO4 Decon 2a
Rhod1hr 11367-152-10 tp4 PO4 Decon 3
Rhod1hr 11367-152-10 tp4 PO4 Decon 3a
Rhod5min 11367-152-10 tp4 PO4 Decon 1
Rhod5min 11367-152-10 tp4 PO4 Decon 1a
Rhod5min 11367-152-10 tp4 PO4 Decon 2
Rhod5min 11367-152-10 tp4 PO4 Decon 2a
Rhod5min 11367-152-10 tp4 PO4 Decon 3
Rhod5min 11367-152-10 tp4 PO4 Decon 3a
MethBlue 11367-152-10 tp4 PO4 Decon 1
BF Rhod1hr 11367-152-10 tp4 PO4 Decon 3
BF Rhod5min 11367-152-10 tp4 PO4 Decon 3
BF MethBlue 11367-152-10 tp4 PO4 Decon 3
BF NoStain 11367-152-10 tp4 PO4 Decon 1
Female AA, individual 11367-152-10 at time point 4 after phosphate buffer decontamination.

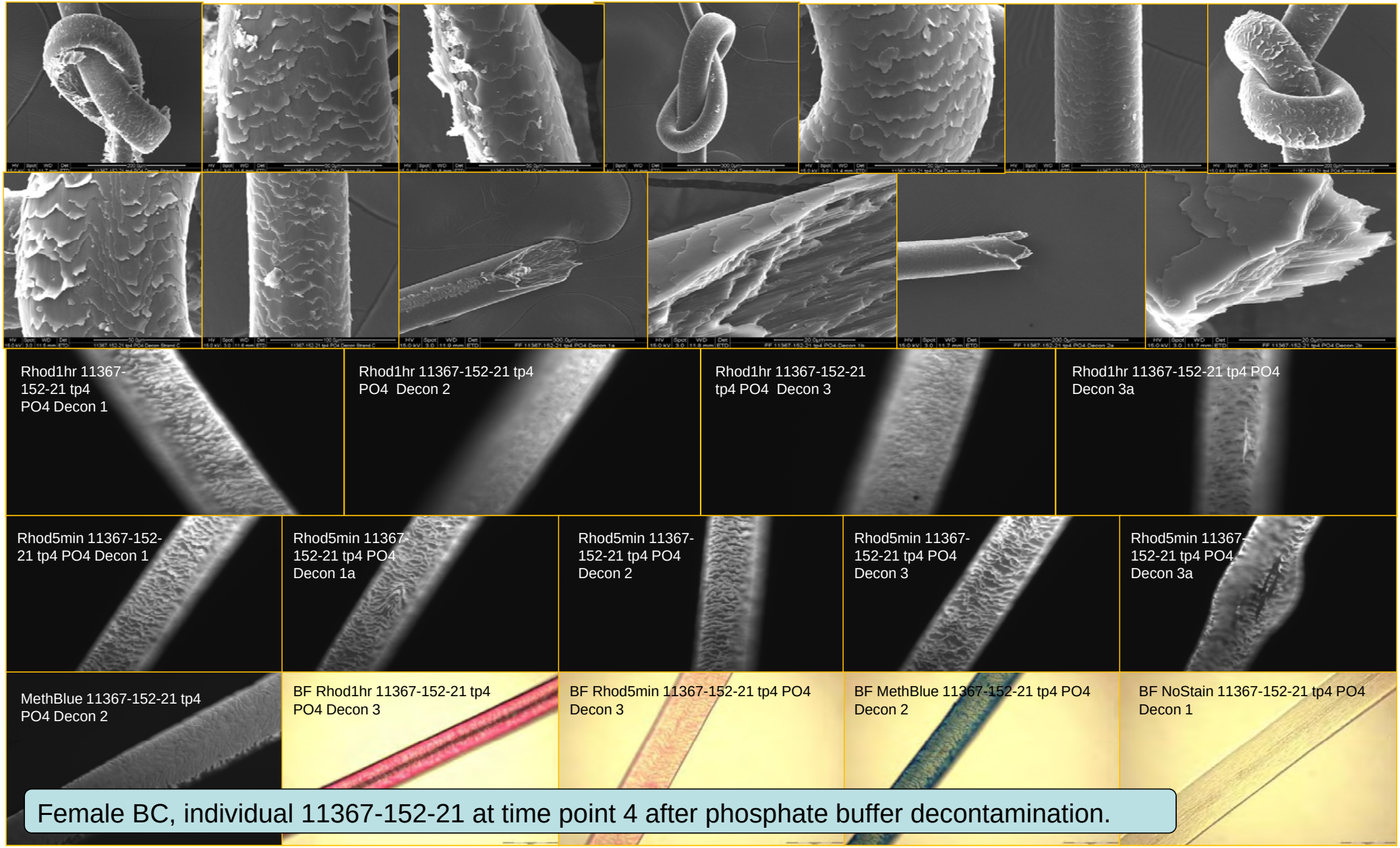


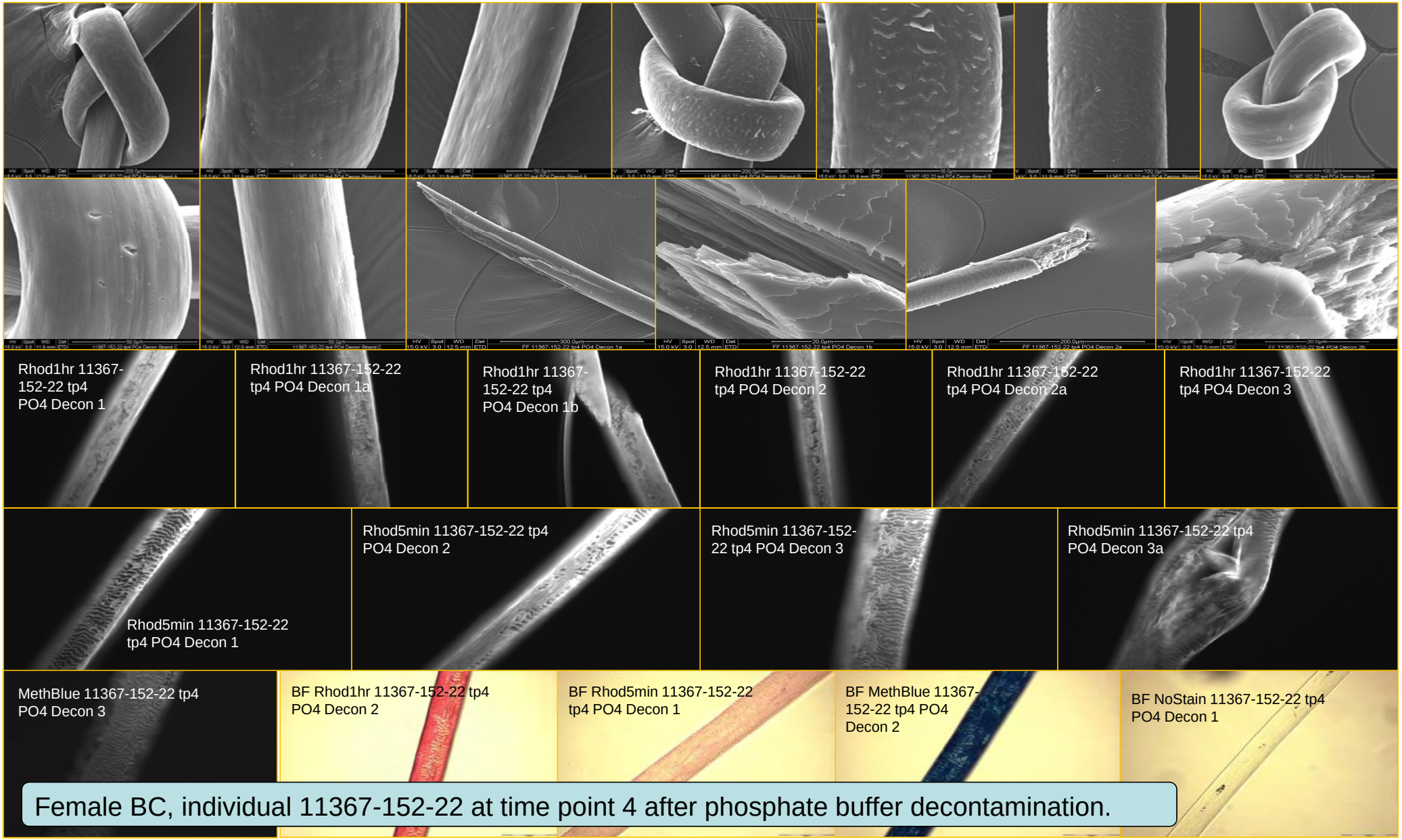
Male AA, individual 11367-152-37 at time point 4 after phosphate buffer decontamination.

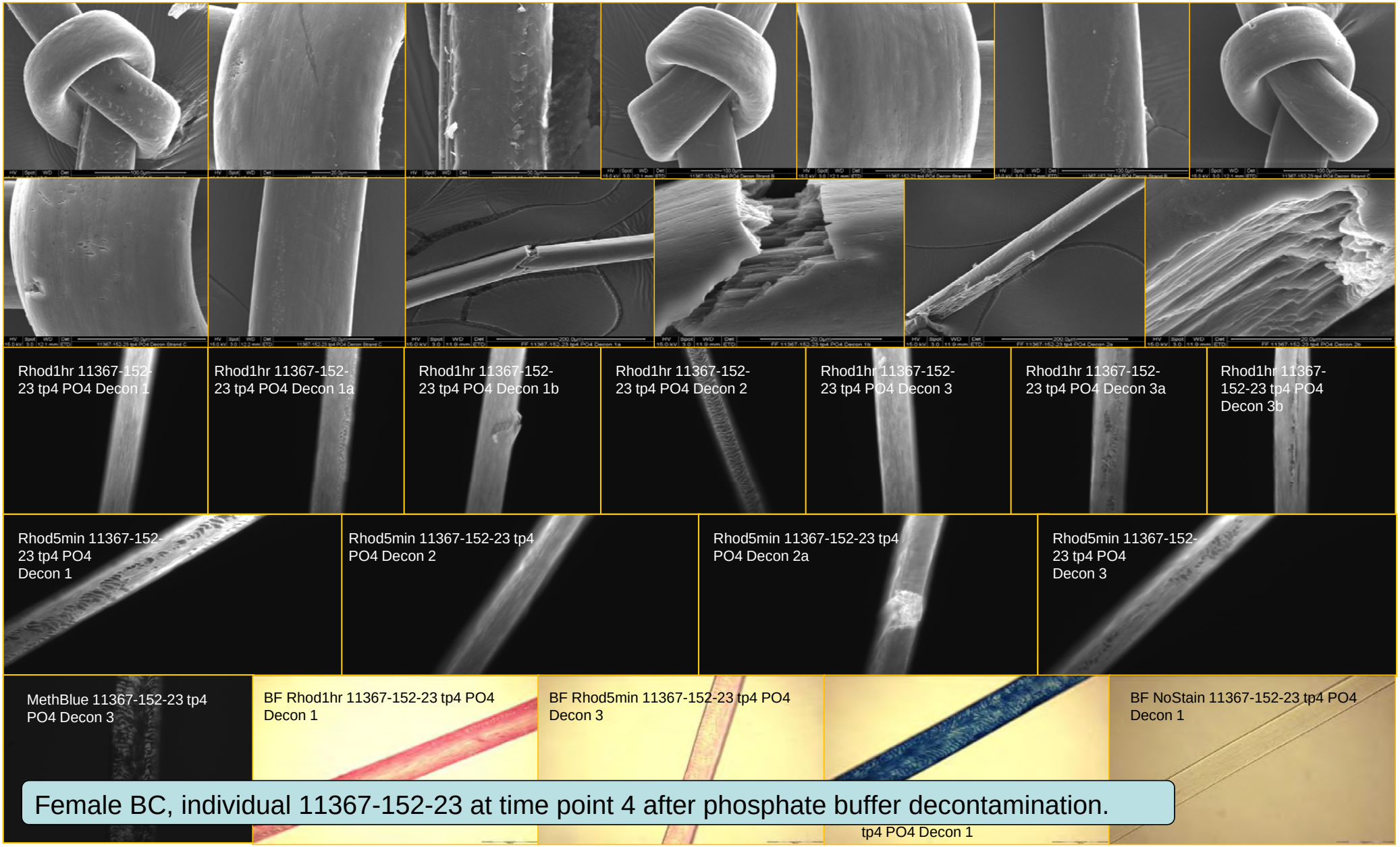

Rhod1hr 11367-152-45 tp4 PO4 Decon 1
Rhod1hr 11367-152-45 tp4 PO4 Decon 1a
Rhod1hr 11367-152-45 tp4 PO4 Decon 2
Rhod1hr 11367-152-45 tp4 PO4 Decon 2a
Rhod1hr 11367-152-45 tp4 PO4 Decon 3
Rhod1hr 11367-152-45 tp4 PO4 Decon 3a
Rhod5min 11367-152-45 tp4 PO4 Decon 1
Rhod5min 11367-152-45 tp4 PO4 Decon 2
Rhod5min 11367-152-45 tp4 PO4 Decon 3
MethBlue 11367-152-45 tp4 PO4 Decon 2
BF Rhod1hr 11367-152-45 tp4 PO4 Decon 3
BF Rhod5min 11367-152-45 tp4 PO4 Decon 2
BF MethBlue 11367-152-45 tp4 PO4 Decon 1
BF NoStain 11367-152-45 tp4 PO4 Decon 1
Female AA, individual 11367-152-45 at time point 4 after phosphate buffer decontamination.

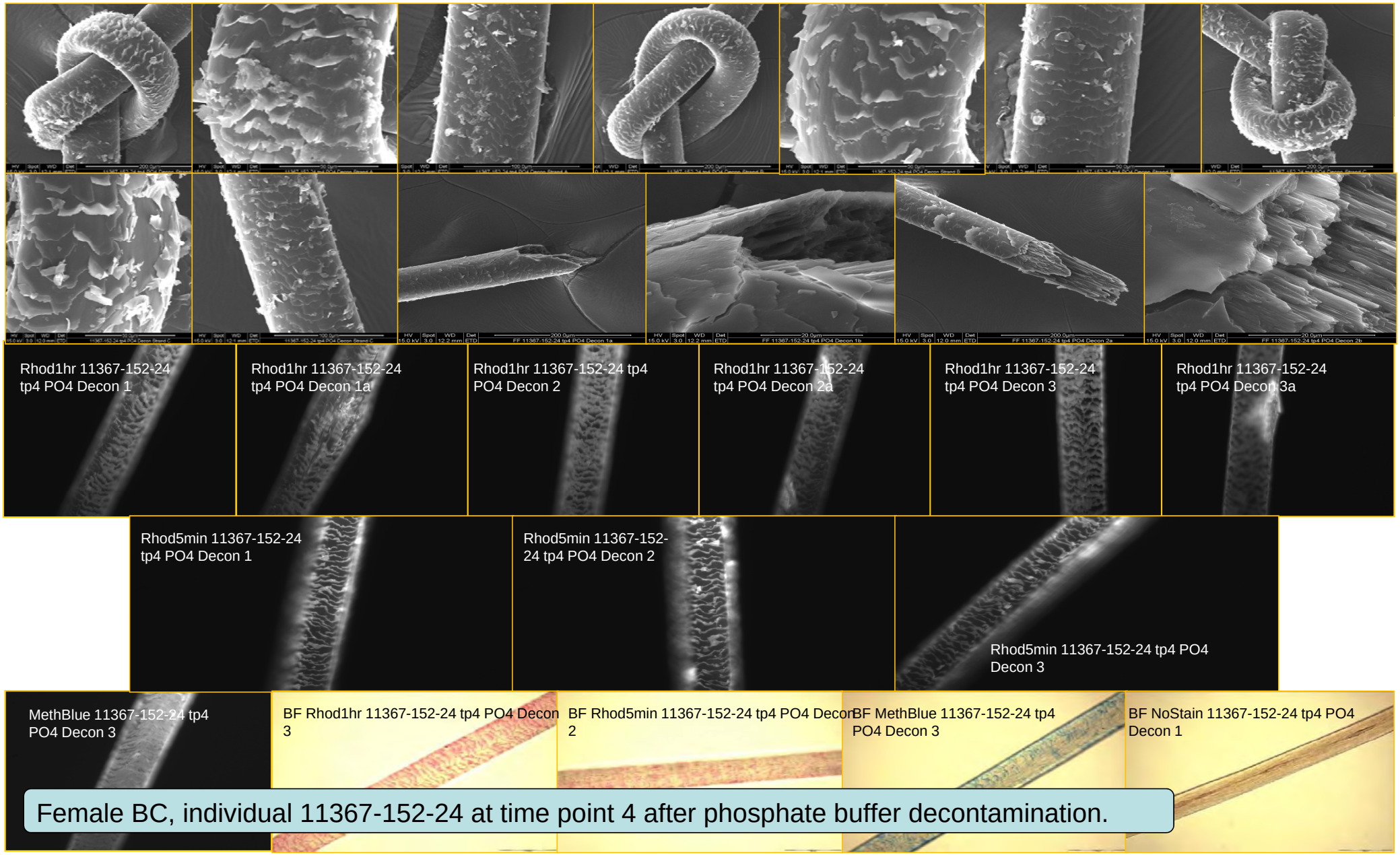

Rhod1hr 11367-152-49 tp4 PO4 Decon 1
Rhod1hr 11367-152-49 tp4 PO4 Decon 2
Rhod1hr 11367-152-49 tp4 PO4 Decon 3
Rhod1hr 11367-152-49 tp4 PO4 Decon 3a
Rhod1hr 11367-152-49 tp4 PO4 Decon 3b
Rhod5min 11367-152-49 tp4 PO4 Decon 1
Rhod5min 11367-152-49 tp4 PO4 Decon 1a
Rhod5min 11367-152-49 tp4 PO4 Decon 2
Rhod5min 11367-152-49 tp4 PO4 Decon 3
MethBlue 11367-152-49 tp4 PO4 Decon 1
BF Rhod1hr 11367-152-49 tp4 PO4 Decon 3
BF Rhod5min 11367-152-49 tp4 PO4 Decon 2
BF MethBlue 11367-152-49 tp4 PO4 Decon 3
BF NoStain 11367-152-49 tp4 PO4 Decon 1
Male AA, individual 11367-152-49 at time point 4 after phosphate buffer decontamination.

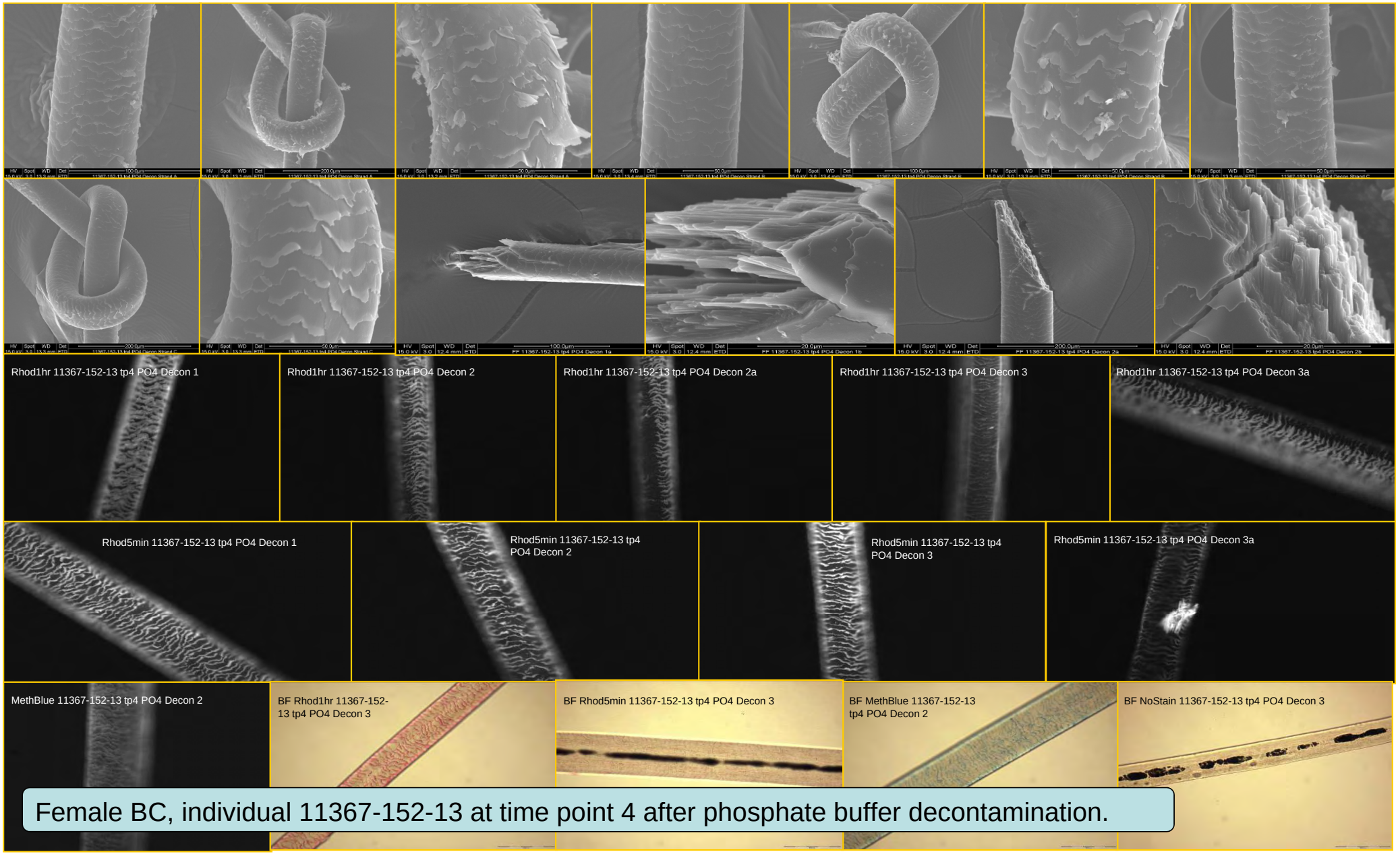

Rhod1hr 11367-152-50 tp4 PO4 Decon 1
Rhod1hr 11367-152-50 tp4 PO4 Decon 2
Rhod1hr 11367-152-50 tp4 PO4 Decon 3
Rhod5min 11367-152-50 tp4 PO4 Decon 1
Rhod5min 11367-152-50 tp4 PO4 Decon 1a
Rhod5min 11367-152-50 tp4 PO4 Decon 2
Rhod5min 11367-152-50 tp4 PO4 Decon 3
MethBlue 11367-152-50 tp4 PO4 Decon 3
BF Rhod1hr 11367-152-50 tp4 PO4 Decon 3
BF MethBlue 11367-152-50 tp4 PO4 Decon 1
BF NoStain 11367-152-50 tp4 PO4 Decon 2
Female AA, individual 11367-152-50 at time point 4 after phosphate buffer decontamination.

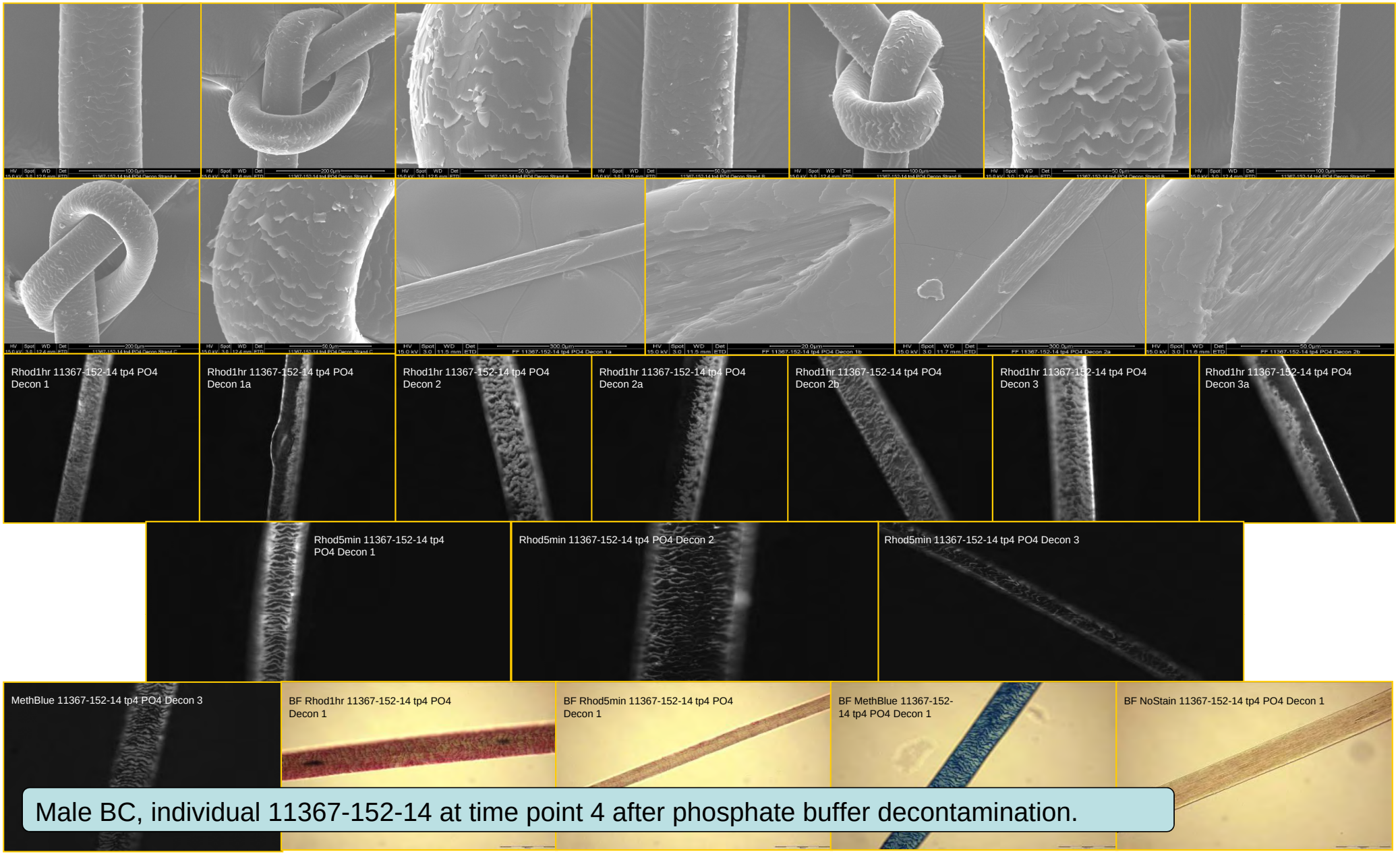

Rhod1hr 11367-152-21 tp4 PO4 Decon 1
Rhod1hr 11367-152-21 tp4 PO4 Decon 2
Rhod1hr 11367-152-21 tp4 PO4 Decon 3
Rhod1hr 11367-152-21 tp4 PO4 Decon 3a
Rhod5min 11367-152-21 tp4 PO4 Decon 1
Rhod5min 11367-152-21 tp4 PO4 Decon 1a
Rhod5min 11367-152-21 tp4 PO4 Decon 2
Rhod5min 11367-152-21 tp4 PO4 Decon 3
Rhod5min 11367-152-21 tp4 PO4 Decon 3a
MethBlue 11367-152-21 tp4 PO4 Decon 2
BF Rhod1hr 11367-152-21 tp4 PO4 Decon 3
BF Rhod5min 11367-152-21 tp4 PO4 Decon 3
BF MethBlue 11367-152-21 tp4 PO4 Decon 2
BF NoStain 11367-152-21 tp4 PO4 Decon 1
Female BC, individual 11367-152-21 at time point 4 after phosphate buffer decontamination.

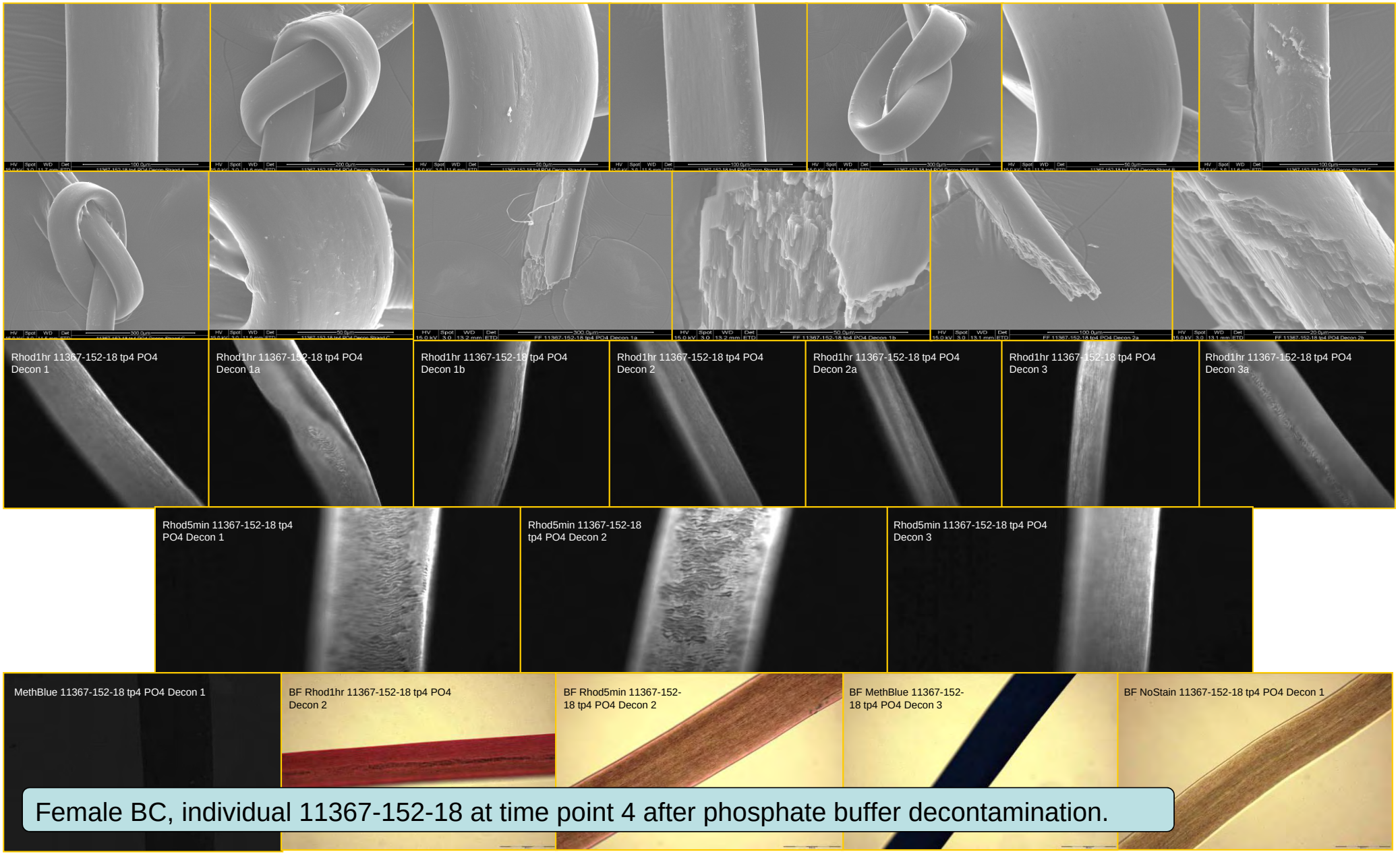

Rhod1hr 11367-152-22 tp4 PO4 Decon 1
Rhod1hr 11367-152-22 tp4 PO4 Decon 1a
Rhod1hr 11367-152-22 tp4 PO4 Decon 1b
Rhod1hr 11367-152-22 tp4 PO4 Decon 2
Rhod1hr 11367-152-22 tp4 PO4 Decon 2a
Rhod1hr 11367-152-22 tp4 PO4 Decon 3
Rhod5min 11367-152-22 tp4 PO4 Decon 1
Rhod5min 11367-152-22 tp4 PO4 Decon 2
Rhod5min 11367-152-22 tp4 PO4 Decon 3
Rhod5min 11367-152-22 tp4 PO4 Decon 3a
MethBlue 11367-152-22 tp4 PO4 Decon 3
BF Rhod1hr 11367-152-22 tp4 PO4 Decon 2
BF Rhod5min 11367-152-22 tp4 PO4 Decon 1
BF MethBlue 11367-152-22 tp4 PO4 Decon 2
BF NoStain 11367-152-22 tp4 PO4 Decon 1
Female BC, individual 11367-152-22 at time point 4 after phosphate buffer decontamination.

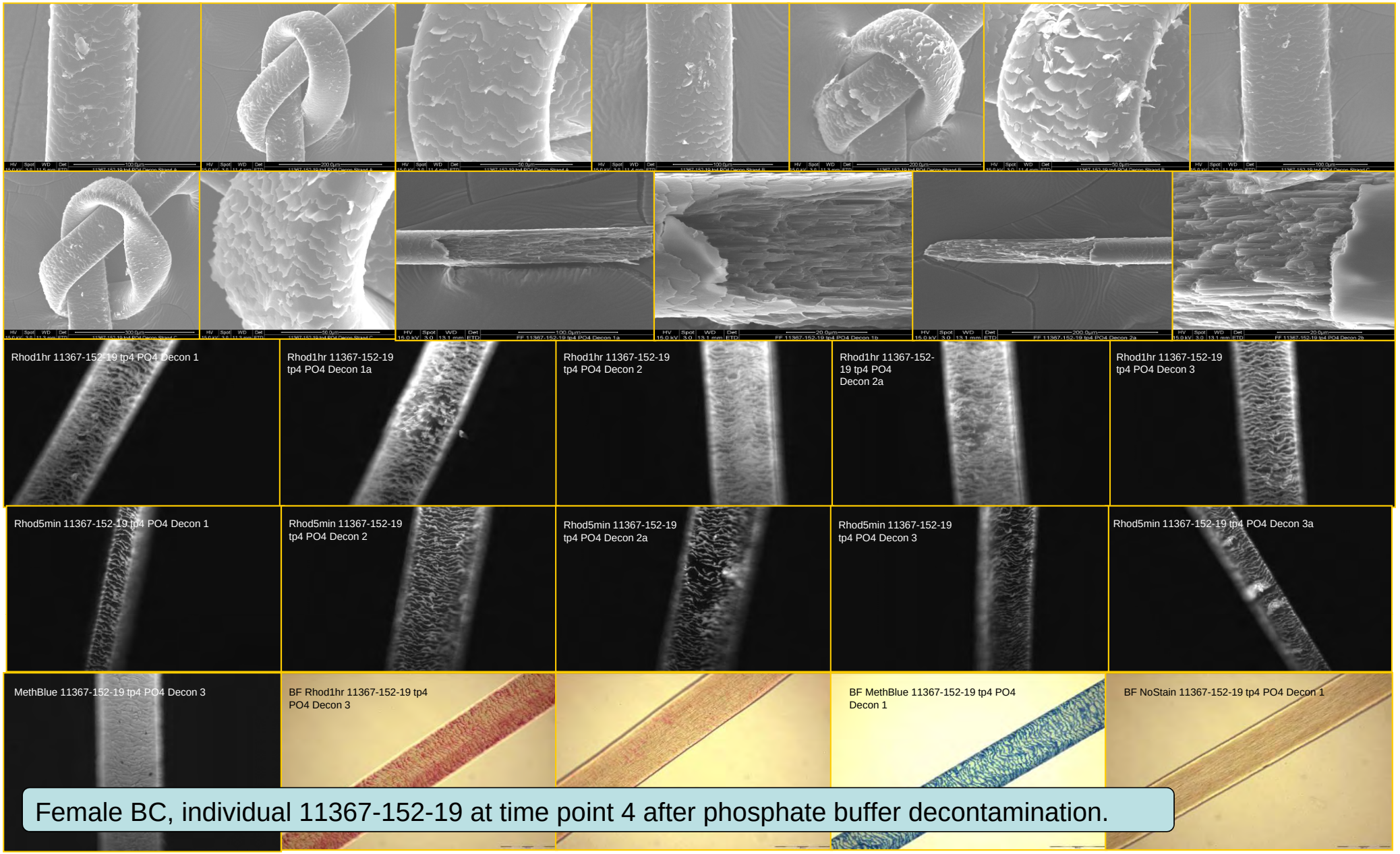

Rhod1hr 11367-152-23 tp4 PO4 Decon 1
Rhod1hr 11367-152-23 tp4 PO4 Decon 1a
Rhod1hr 11367-152-23 tp4 PO4 Decon 1b
Rhod1hr 11367-152-23 tp4 PO4 Decon 2
Rhod1hr 11367-152-23 tp4 PO4 Decon 3
Rhod1hr 11367-152-23 tp4 PO4 Decon 3a
Rhod1hr 11367-152-23 tp4 PO4 Decon 3b
Rhod5min 11367-152-23 tp4 PO4 Decon 1
Rhod5min 11367-152-23 tp4 PO4 Decon 2
Rhod5min 11367-152-23 tp4 PO4 Decon 2a
Rhod5min 11367-152-23 tp4 PO4 Decon 3
MethBlue 11367-152-23 tp4 PO4 Decon 3
BF Rhod1hr 11367-152-23 tp4 PO4 Decon 1
BF Rhod5min 11367-152-23 tp4 PO4 Decon 3
BF NoStain 11367-152-23 tp4 PO4 Decon 1
Female BC, individual 11367-152-23 at time point 4 after phosphate buffer decontamination.
tp4 PO4 Decon 1

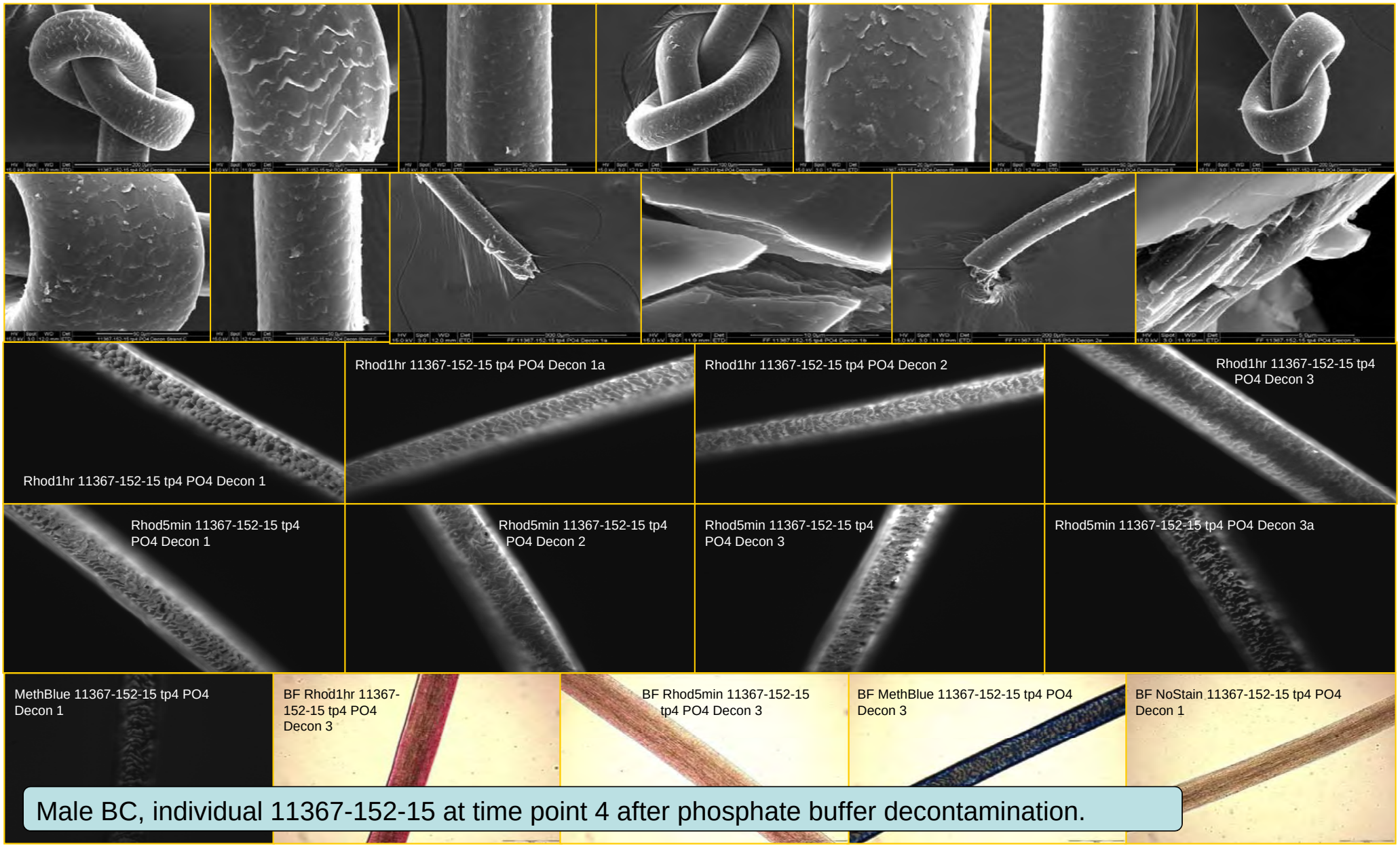

Rhod1hr 11367-152-24 tp4 PO4 Decon 1
Rhod1hr 11367-152-24 tp4 PO4 Decon 1a
Rhod1hr 11367-152-24 tp4 PO4 Decon 2
Rhod1hr 11367-152-24 tp4 PO4 Decon 2a
Rhod1hr 11367-152-24 tp4 PO4 Decon 3
Rhod1hr 11367-152-24 tp4 PO4 Decon 3a
Rhod5min 11367-152-24 tp4 PO4 Decon 1
Rhod5min 11367-152-24 tp4 PO4 Decon 2
Rhod5min 11367-152-24 tp4 PO4 Decon 3
MethBlue 11367-152-24 tp4 PO4 Decon 3
BF Rhod1hr 11367-152-24 tp4 PO4 Decon 3
BF Rhod5min 11367-152-24 tp4 PO4 Decon 2
BF MethBlue 11367-152-24 tp4 PO4 Decon 3
BF NoStain 11367-152-24 tp4 PO4 Decon 1
Female BC, individual 11367-152-24 at time point 4 after phosphate buffer decontamination.

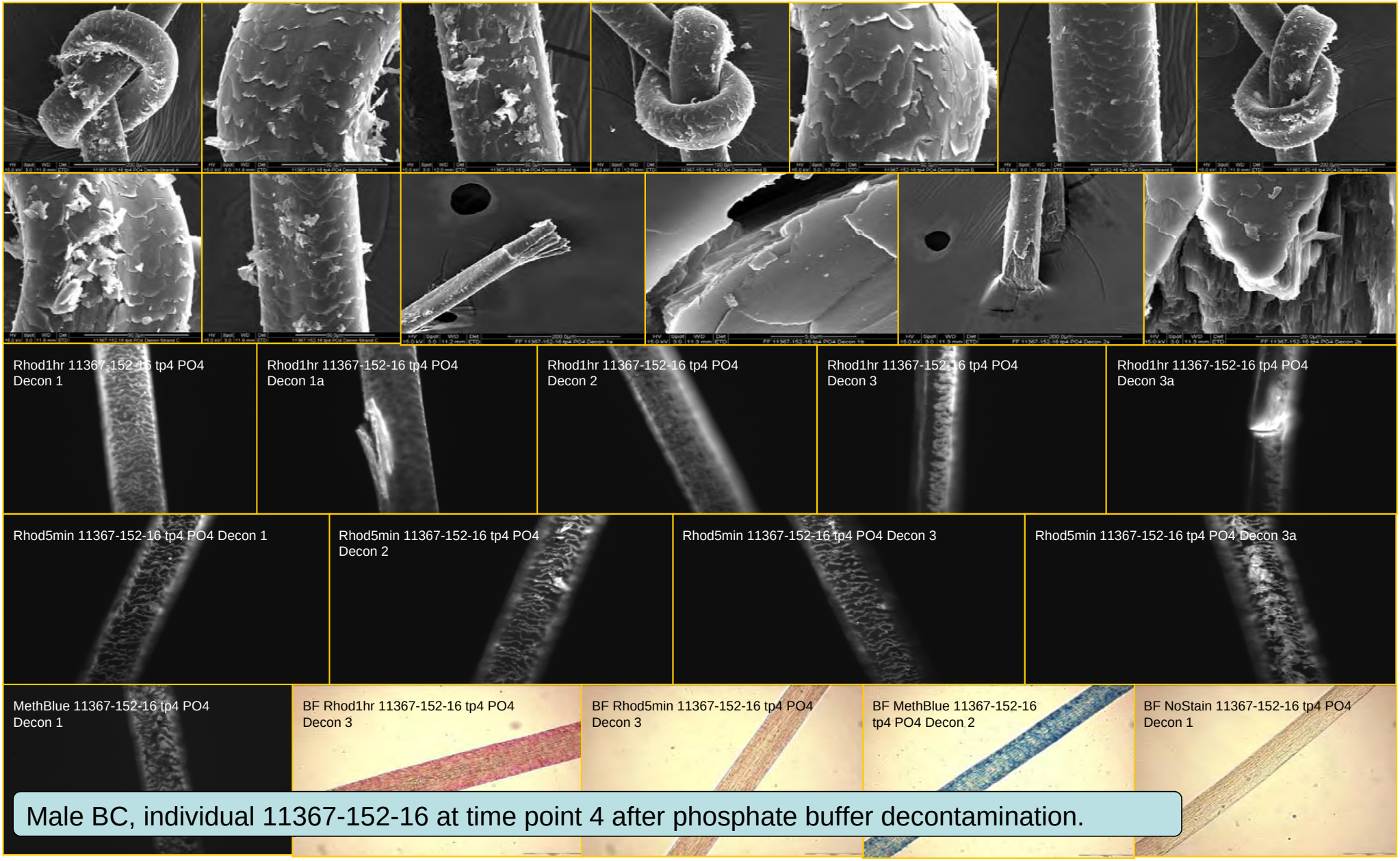

Rhod1hr 11367-152-13 tp4 PO4 Decon 1
Rhod1hr 11367-152-13 tp4 PO4 Decon 2
Rhod1hr 11367-152-13 tp4 PO4 Decon 2a
Rhod1hr 11367-152-13 tp4 PO4 Decon 3
Rhod1hr 11367-152-13 tp4 PO4 Decon 3a
Rhod5min 11367-152-13 tp4 PO4 Decon 1
Rhod5min 11367-152-13 tp4 PO4 Decon 2
Rhod5min 11367-152-13 tp4 PO4 Decon 3
Rhod5min 11367-152-13 tp4 PO4 Decon 3a
MethBlue 11367-152-13 tp4 PO4 Decon 2
BF Rhod1hr 11367-152-13 tp4 PO4 Decon 3
BF Rhod5min 11367-152-13 tp4 PO4 Decon 3
BF MethBlue 11367-152-13 tp4 PO4 Decon 2
BF NoStain 11367-152-13 tp4 PO4 Decon 3
Female BC, individual 11367-152-13 at time point 4 after phosphate buffer decontamination.

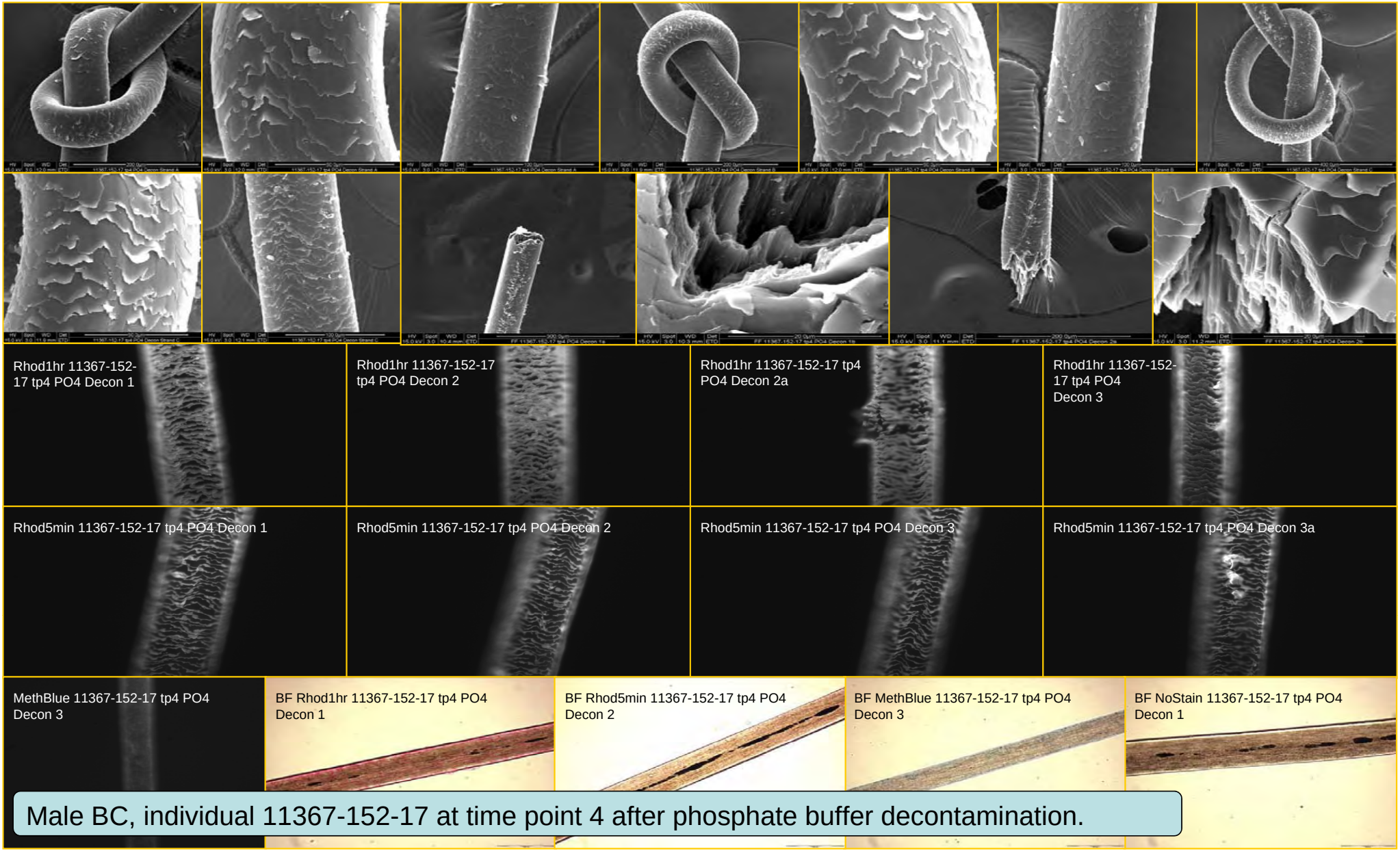

Rhod1hr 11367-152-14 tp4 PO4 Decon 1
Rhod1hr 11367-152-14 tp4 PO4 Decon 1a
Rhod1hr 11367-152-14 tp4 PO4 Decon 2
Rhod1hr 11367-152-14 tp4 PO4 Decon 2a
Rhod1hr 11367-152-14 tp4 PO4 Decon 2b
Rhod1hr 11367-152-14 tp4 PO4 Decon 3
Rhod1hr 11367-152-14 tp4 PO4 Decon 3a
Rhod5min 11367-152-14 tp4 PO4 Decon 1
Rhod5min 11367-152-14 tp4 PO4 Decon 2
Rhod5min 11367-152-14 tp4 PO4 Decon 3
MethBlue 11367-152-14 tp4 PO4 Decon 3
BF Rhod1hr 11367-152-14 tp4 PO4 Decon 1
BF Rhod5min 11367-152-14 tp4 PO4 Decon 1
BF MethBlue 11367-152-14 tp4 PO4 Decon 1
BF NoStain 11367-152-14 tp4 PO4 Decon 1
Male BC, individual 11367-152-14 at time point 4 after phosphate buffer decontamination.

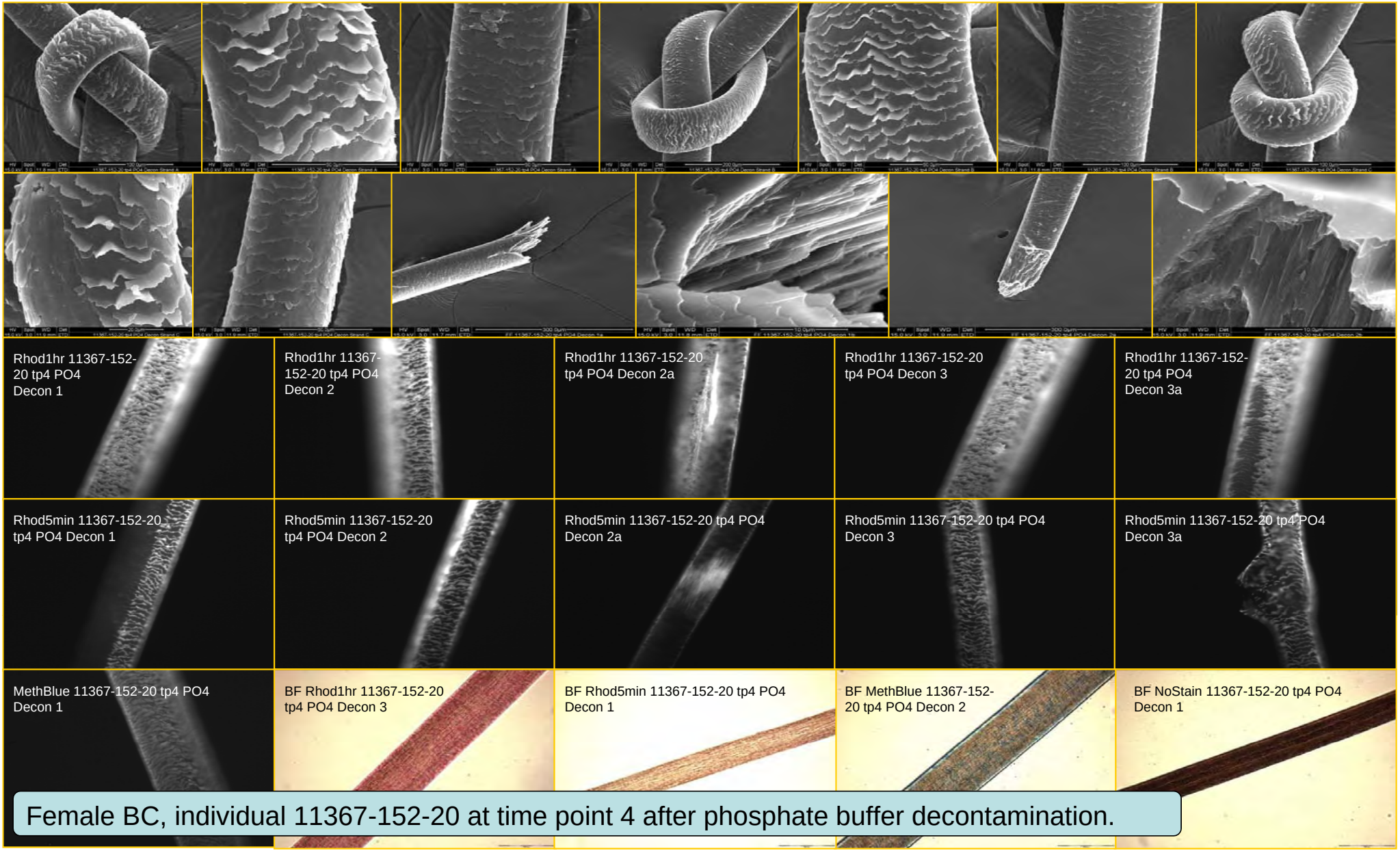

Rhod1hr 11367-152-18 tp4 PO4 Decon 1
Rhod1hr 11367-152-18 tp4 PO4 Decon 1a
Rhod1hr 11367-152-18 tp4 PO4 Decon 1b
Rhod1hr 11367-152-18 tp4 PO4 Decon 2
Rhod1hr 11367-152-18 tp4 PO4 Decon 2a
Rhod1hr 11367-152-18 tp4 PO4 Decon 3
Rhod1hr 11367-152-18 tp4 PO4 Decon 3a
Rhod5min 11367-152-18 tp4 PO4 Decon 1
Rhod5min 11367-152-18 tp4 PO4 Decon 2
Rhod5min 11367-152-18 tp4 PO4 Decon 3
MethBlue 11367-152-18 tp4 PO4 Decon 1
BF Rhod1hr 11367-152-18 tp4 PO4 Decon 2
BF Rhod5min 11367-152-18 tp4 PO4 Decon 2
BF MethBlue 11367-152-18 tp4 PO4 Decon 3
BF NoStain 11367-152-18 tp4 PO4 Decon 1
Female BC, individual 11367-152-18 at time point 4 after phosphate buffer decontamination.

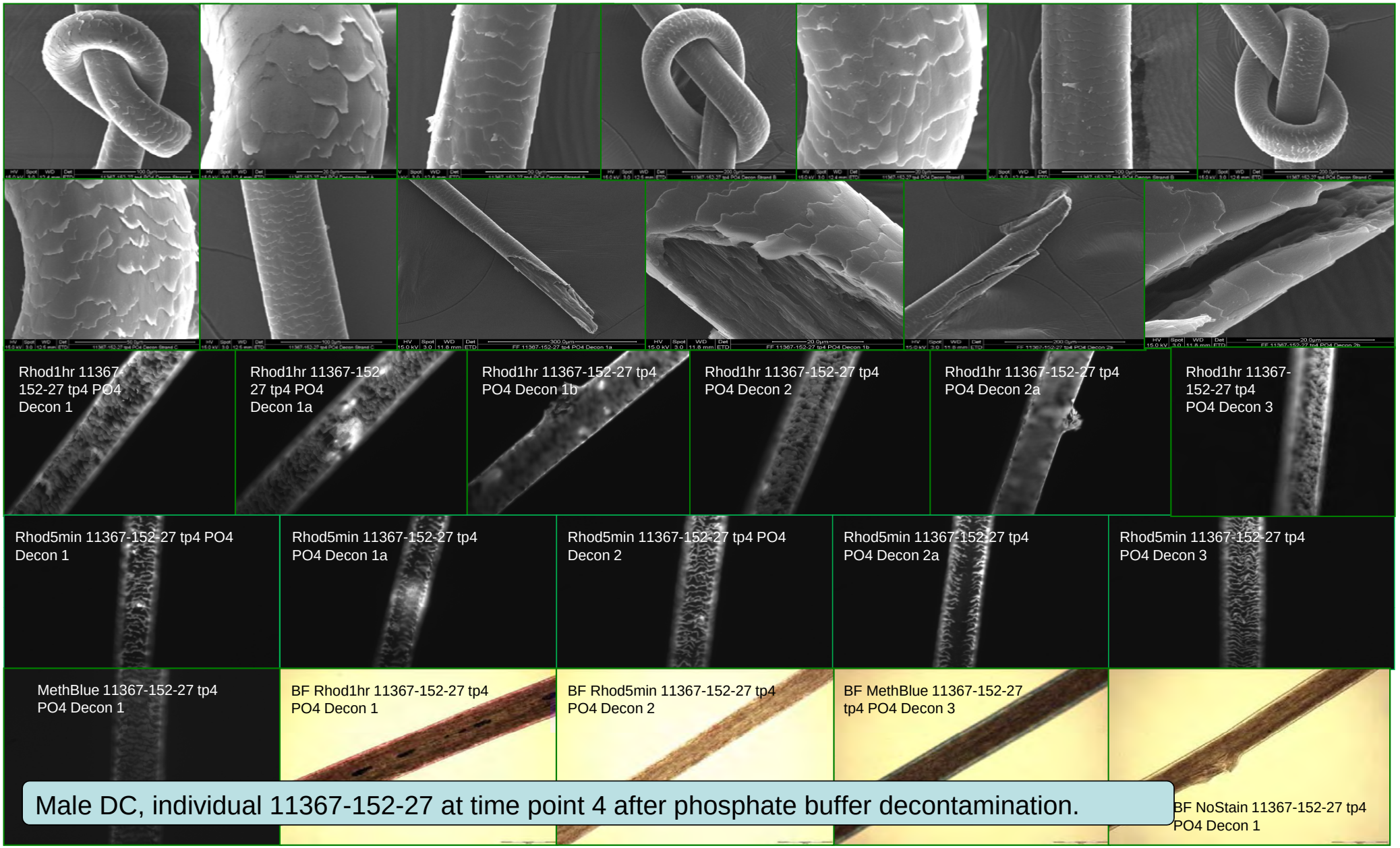

Rhod1hr 11367-152-19 tp4 PO4 Decon 1
Rhod1hr 11367-152-19 tp4 PO4 Decon 1a
Rhod1hr 11367-152-19 tp4 PO4 Decon 2
Rhod1hr 11367-152-19 tp4 PO4 Decon 2a
Rhod1hr 11367-152-19 tp4 PO4 Decon 3
Rhod5min 11367-152-19 tp4 PO4 Decon 1
Rhod5min 11367-152-19 tp4 PO4 Decon 2
Rhod5min 11367-152-19 tp4 PO4 Decon 2a
Rhod5min 11367-152-19 tp4 PO4 Decon 3
Rhod5min 11367-152-19 tp4 PO4 Decon 3a
MethBlue 11367-152-19 tp4 PO4 Decon 3
BF Rhod1hr 11367-152-19 tp4 PO4 Decon 3
BF MethBlue 11367-152-19 tp4 PO4 Decon 1
BF NoStain 11367-152-19 tp4 PO4 Decon 1
Female BC, individual 11367-152-19 at time point 4 after phosphate buffer decontamination.


Rhod1hr 11367-152-15 tp4 PO4 Decon 1
Rhod1hr 11367-152-15 tp4 PO4 Decon 1a
Rhod1hr 11367-152-15 tp4 PO4 Decon 2
Rhod1hr 11367-152-15 tp4 PO4 Decon 3
Rhod5min 11367-152-15 tp4 PO4 Decon 1
Rhod5min 11367-152-15 tp4 PO4 Decon 2
Rhod5min 11367-152-15 tp4 PO4 Decon 3
Rhod5min 11367-152-15 tp4 PO4 Decon 3a
MethBlue 11367-152-15 tp4 PO4 Decon 1
BF Rhod1hr 11367-152-15 tp4 PO4 Decon 3
BF Rhod5min 11367-152-15 tp4 PO4 Decon 3
BF MethBlue 11367-152-15 tp4 PO4 Decon 3
BF NoStain 11367-152-15 tp4 PO4 Decon 1
Male BC, individual 11367-152-15 at time point 4 after phosphate buffer decontamination.

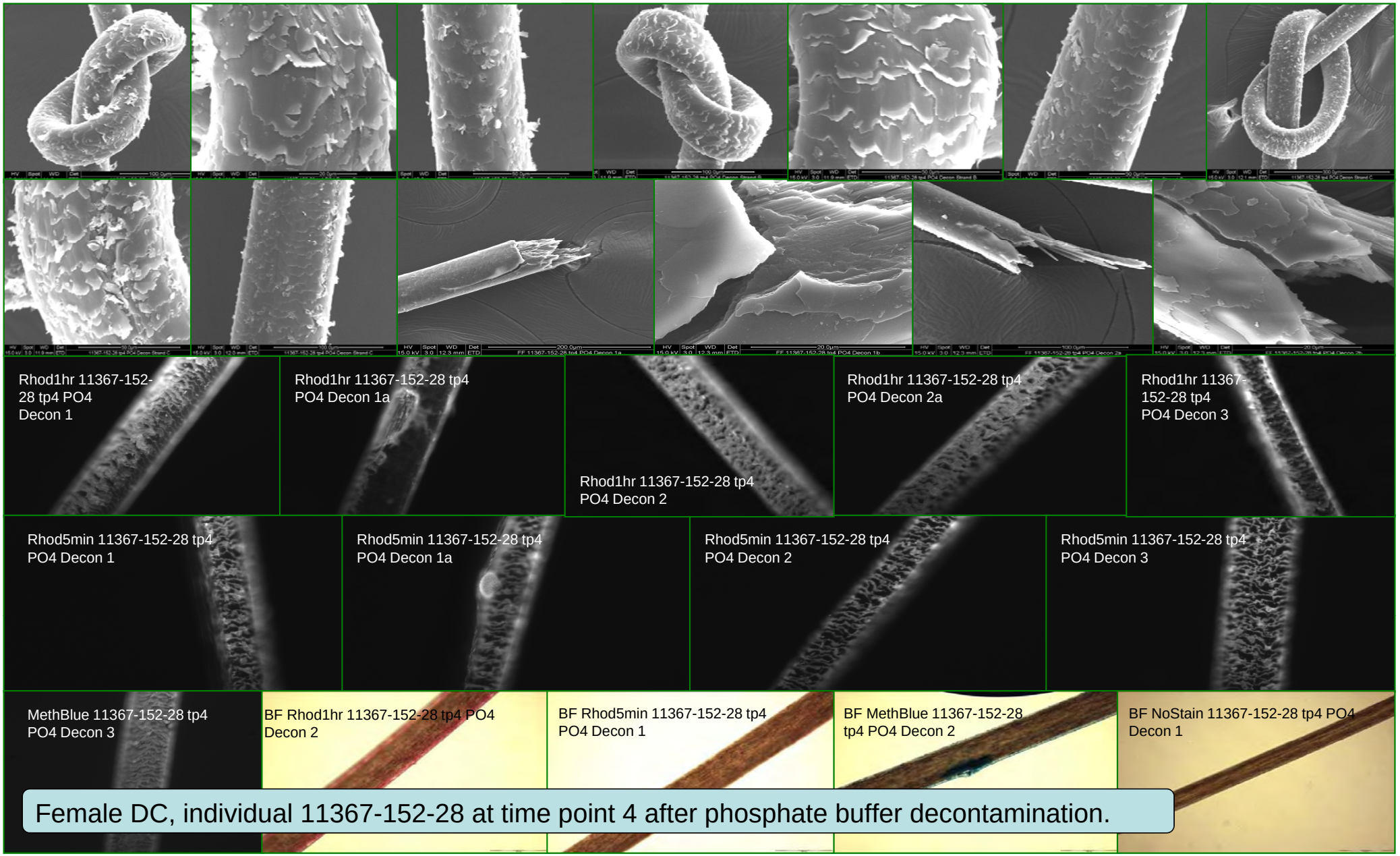

Rhod1hr 11367-152-16 tp4 PO4 Decon 1
Rhod1hr 11367-152-16 tp4 PO4 Decon 1a
Rhod1hr 11367-152-16 tp4 PO4 Decon 2
Rhod1hr 11367-152-16 tp4 PO4 Decon 3
Rhod1hr 11367-152-16 tp4 PO4 Decon 3a
Rhod5min 11367-152-16 tp4 PO4 Decon 1
Rhod5min 11367-152-16 tp4 PO4 Decon 2
Rhod5min 11367-152-16 tp4 PO4 Decon 3
Rhod5min 11367-152-16 tp4 PO4 Decon 3a
MethBlue 11367-152-16 tp4 PO4 Decon 1
BF Rhod1hr 11367-152-16 tp4 PO4 Decon 3
BF Rhod5min 11367-152-16 tp4 PO4 Decon 3
BF MethBlue 11367-152-16 tp4 PO4 Decon 2
BF NoStain 11367-152-16 tp4 PO4 Decon 1
Male BC, individual 11367-152-16 at time point 4 after phosphate buffer decontamination.

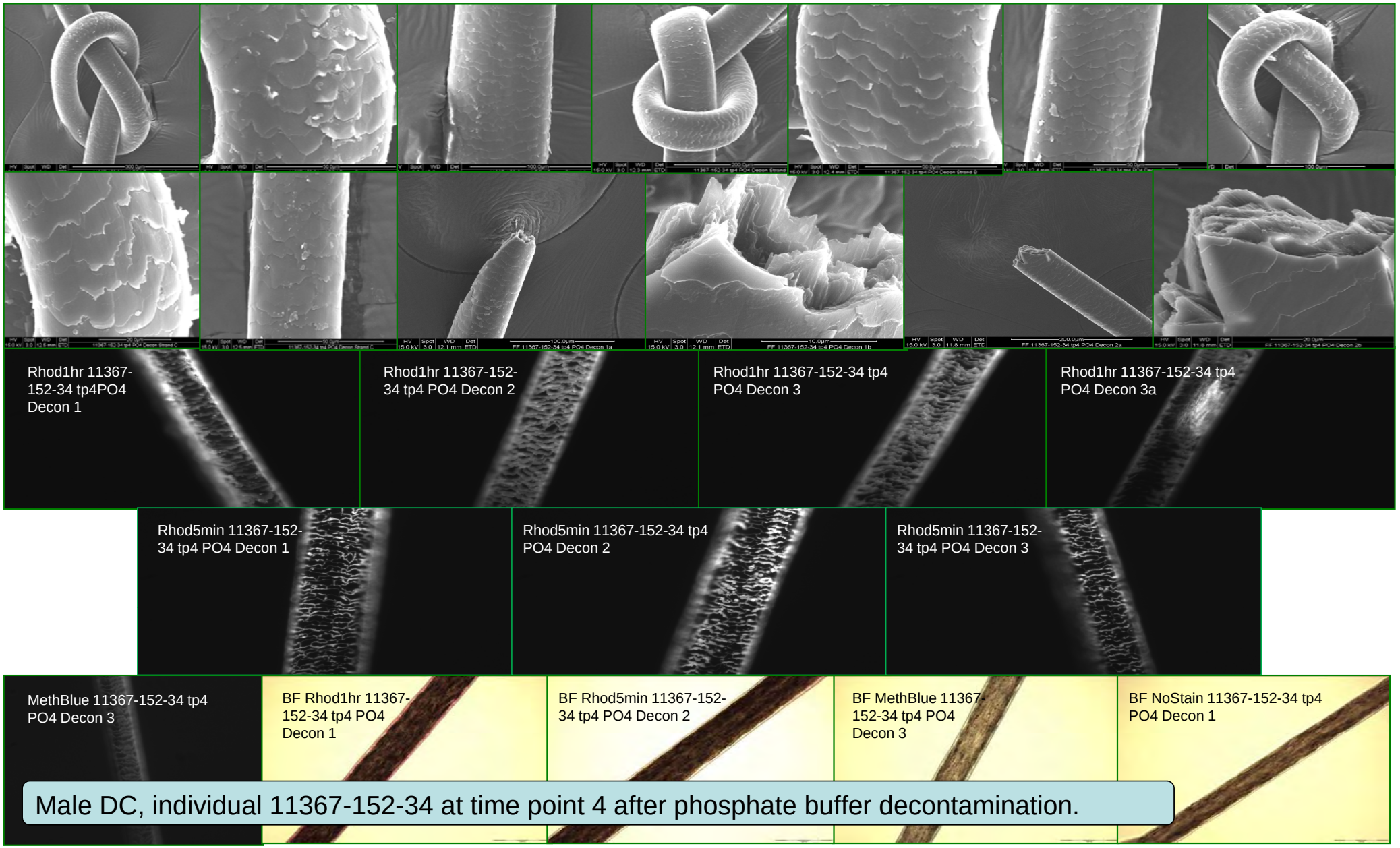

Rhod1hr 11367-152-17 tp4 PO4 Decon 1
Rhod1hr 11367-152-17 tp4 PO4 Decon 2
Rhod1hr 11367-152-17 tp4 PO4 Decon 2a
Rhod1hr 11367-152-17 tp4 PO4 Decon 3
Rhod5min 11367-152-17 tp4 PO4 Decon 1
Rhod5min 11367-152-17 tp4 PO4 Decon 2
Rhod5min 11367-152-17 tp4 PO4 Decon 3
Rhod5min 11367-152-17 tp4 PO4 Decon 3a
MethBlue 11367-152-17 tp4 PO4 Decon 3
BF Rhod1hr 11367-152-17 tp4 PO4 Decon 1
BF Rhod5min 11367-152-17 tp4 PO4 Decon 2
BF MethBlue 11367-152-17 tp4 PO4 Decon 3
BF NoStain 11367-152-17 tp4 PO4 Decon 1
Male BC, individual 11367-152-17 at time point 4 after phosphate buffer decontamination.


Rhod1hr 11367-152-20 tp4 PO4 Decon 1
Rhod1hr 11367-152-20 tp4 PO4 Decon 2
Rhod1hr 11367-152-20 tp4 PO4 Decon 2a
Rhod1hr 11367-152-20 tp4 PO4 Decon 3
Rhod1hr 11367-152-20 tp4 PO4 Decon 3a
Rhod5min 11367-152-20 tp4 PO4 Decon 1
Rhod5min 11367-152-20 tp4 PO4 Decon 2
Rhod5min 11367-152-20 tp4 PO4 Decon 2a
Rhod5min 11367-152-20 tp4 PO4 Decon 3
Rhod5min 11367-152-20 tp4 PO4 Decon 3a
MethBlue 11367-152-20 tp4 PO4 Decon 1
BF Rhod1hr 11367-152-20 tp4 PO4 Decon 3
BF Rhod5min 11367-152-20 tp4 PO4 Decon 1
BF MethBlue 11367-152-20 tp4 PO4 Decon 2
BF NoStain 11367-152-20 tp4 PO4 Decon 1
Female BC, individual 11367-152-20 at time point 4 after phosphate buffer decontamination.

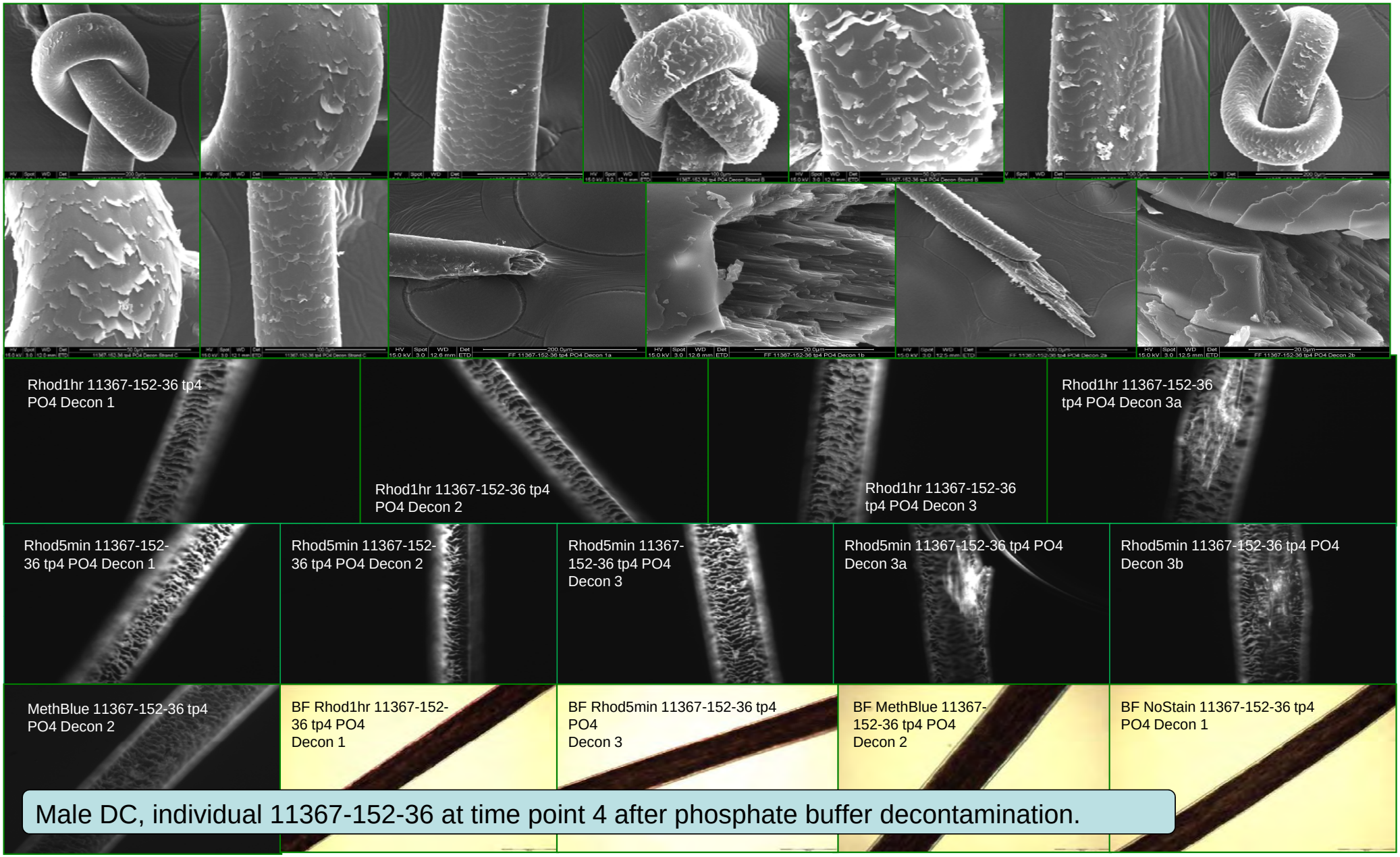


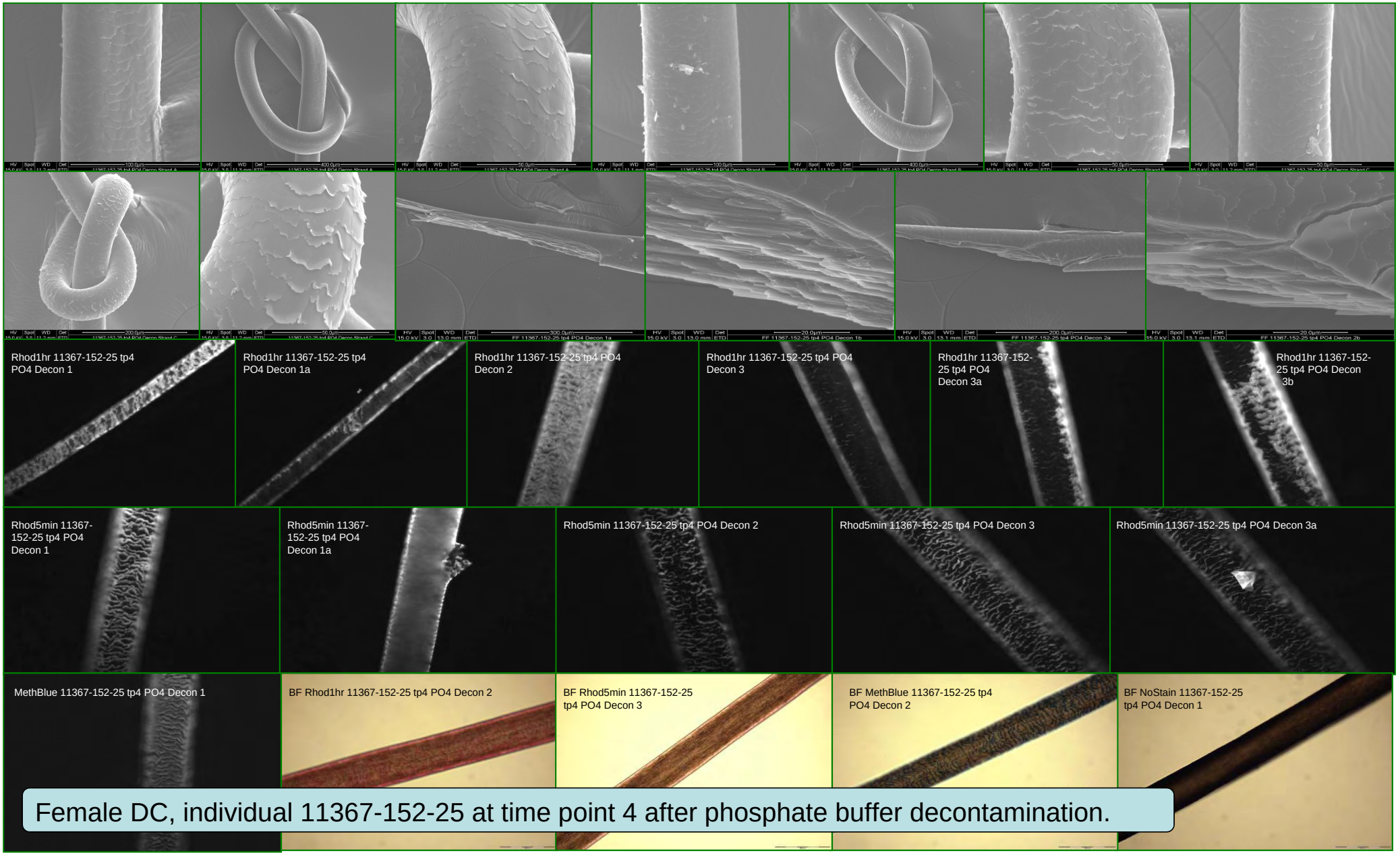
Male DC, individual 11367-152-27 at time point 4 after phosphate buffer decontamination.

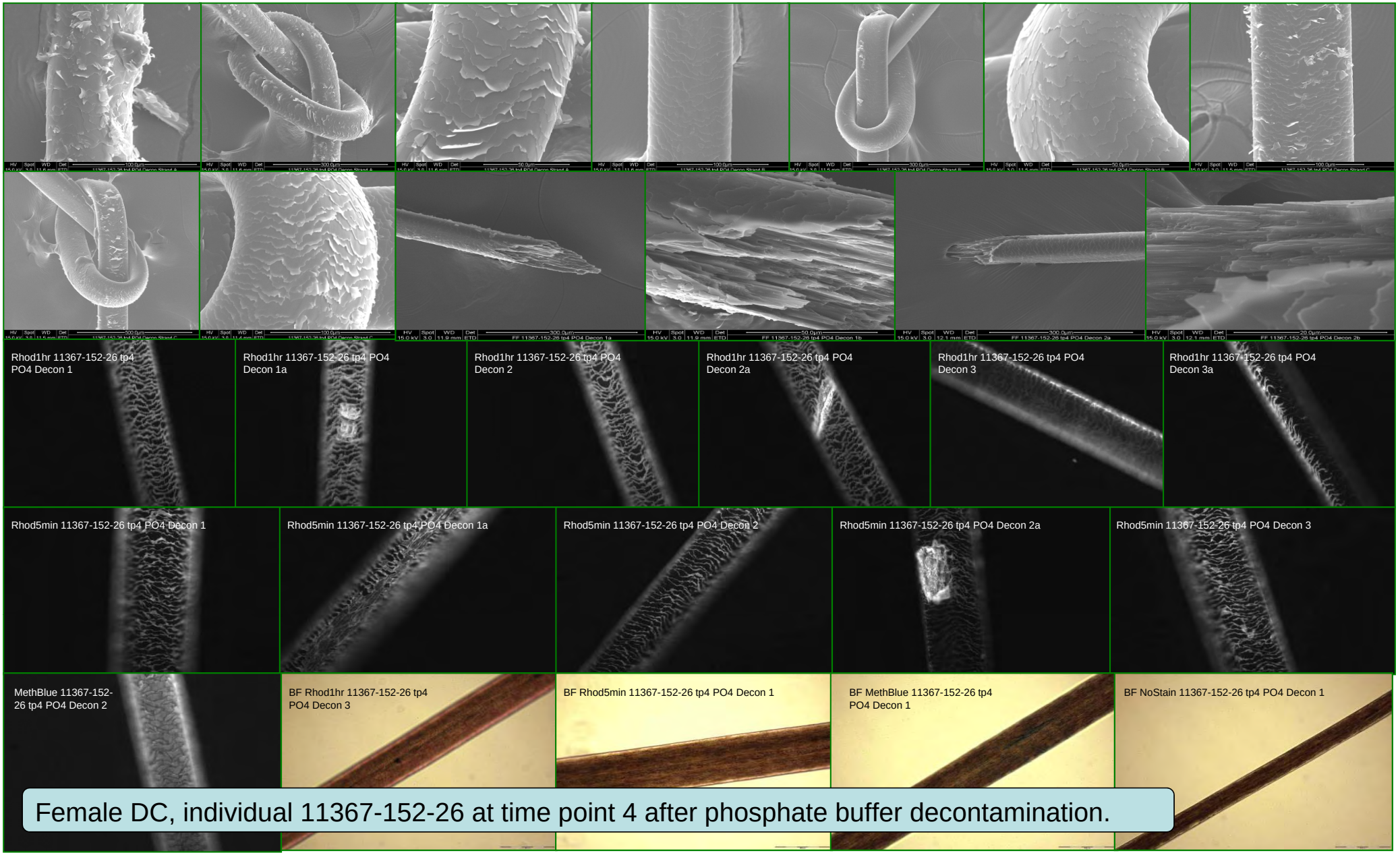

Rhod1hr 11367-152-28 tp4 PO4 Decon 1
Rhod1hr 11367-152-28 tp4 PO4 Decon 1a
Rhod1hr 11367-152-28 tp4 PO4 Decon 2
Rhod1hr 11367-152-28 tp4 PO4 Decon 2a
Rhod1hr 11367-152-28 tp4 PO4 Decon 3
Rhod5min 11367-152-28 tp4 PO4 Decon 1
Rhod5min 11367-152-28 tp4 PO4 Decon 1a
Rhod5min 11367-152-28 tp4 PO4 Decon 2
Rhod5min 11367-152-28 tp4 PO4 Decon 3
MethBlue 11367-152-28 tp4 PO4 Decon 3
BF Rhod1hr 11367-152-28 tp4 PO4 Decon 2
BF Rhod5min 11367-152-28 tp4 PO4 Decon 1
BF MethBlue 11367-152-28 tp4 PO4 Decon 2
BF NoStain 11367-152-28 tp4 PO4 Decon 1
Female DC, individual 11367-152-28 at time point 4 after phosphate buffer decontamination.

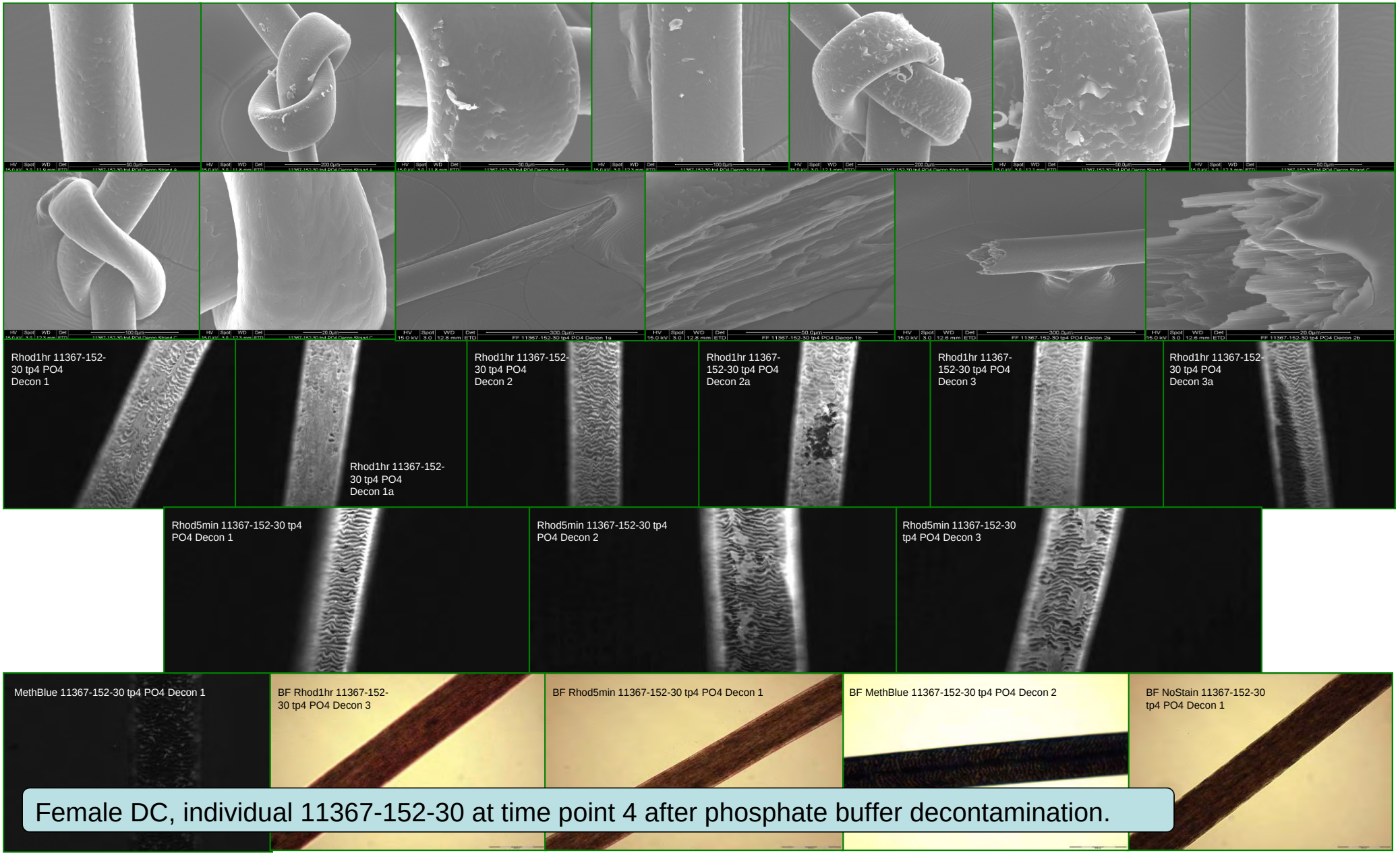


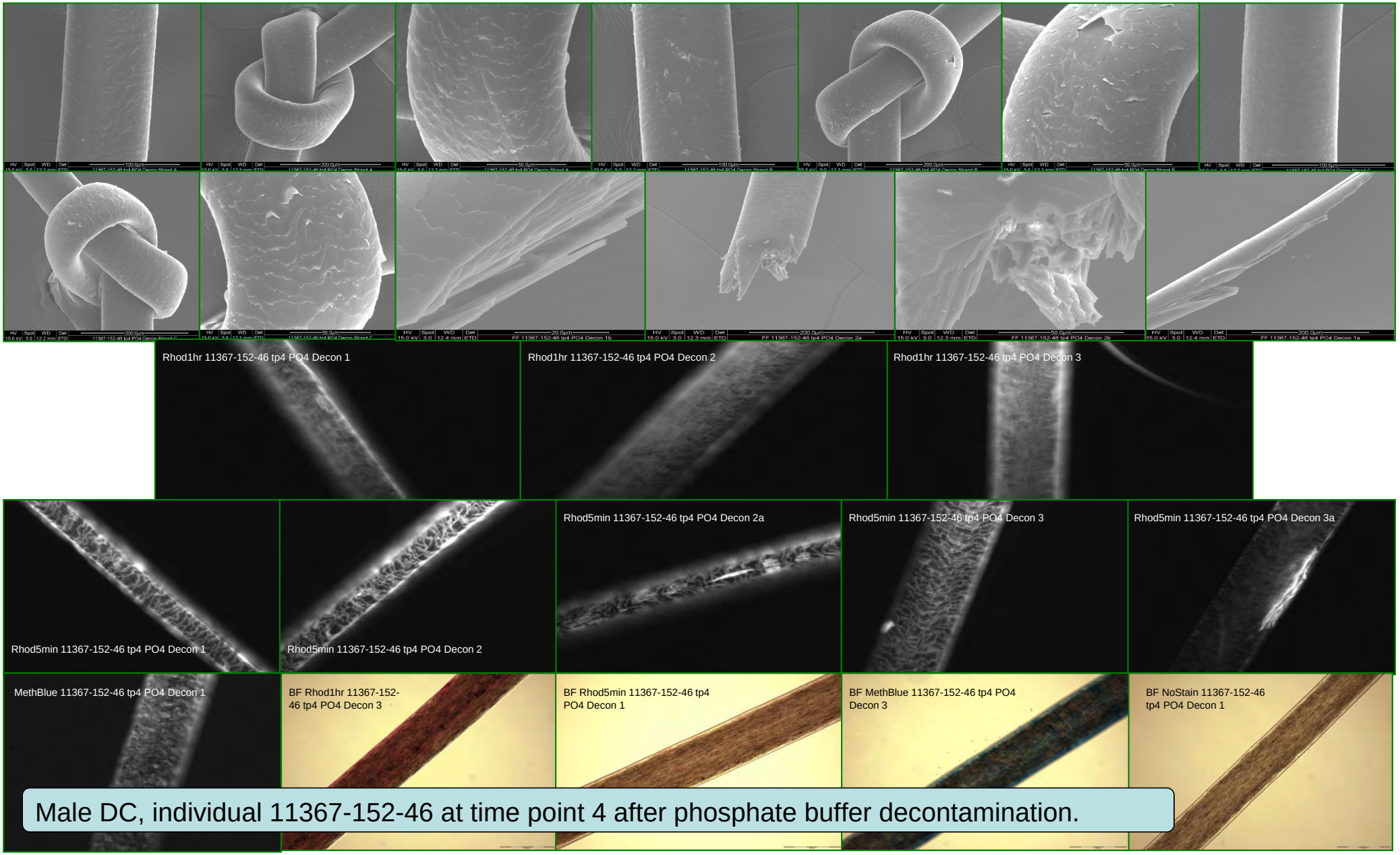
Male DC, individual 11367-152-34 at time point 4 after phosphate buffer decontamination.


Rhod1hr 11367-152-36 tp4 PO4 Decon 1
Rhod1hr 11367-152-36 tp4 PO4 Decon 2
Rhod1hr 11367-152-36 tp4 PO4 Decon 3
Rhod1hr 11367-152-36 tp4 PO4 Decon 3a
Rhod5min 11367-152-36 tp4 PO4 Decon 1
Rhod5min 11367-152-36 tp4 PO4 Decon 2
Rhod5min 11367-152-36 tp4 PO4 Decon 3
Rhod5min 11367-152-36 tp4 PO4 Decon 3a
Rhod5min 11367-152-36 tp4 PO4 Decon 3b
MethBlue 11367-152-36 tp4 PO4 Decon 2
BF Rhod1hr 11367-152-36 tp4 PO4 Decon 1
BF Rhod5min 11367-152-36 tp4 PO4 Decon 3
BF MethBlue 11367-152-36 tp4 PO4 Decon 2
BF NoStain 11367-152-36 tp4 PO4 Decon 1
Male DC, individual 11367-152-36 at time point 4 after phosphate buffer decontamination.

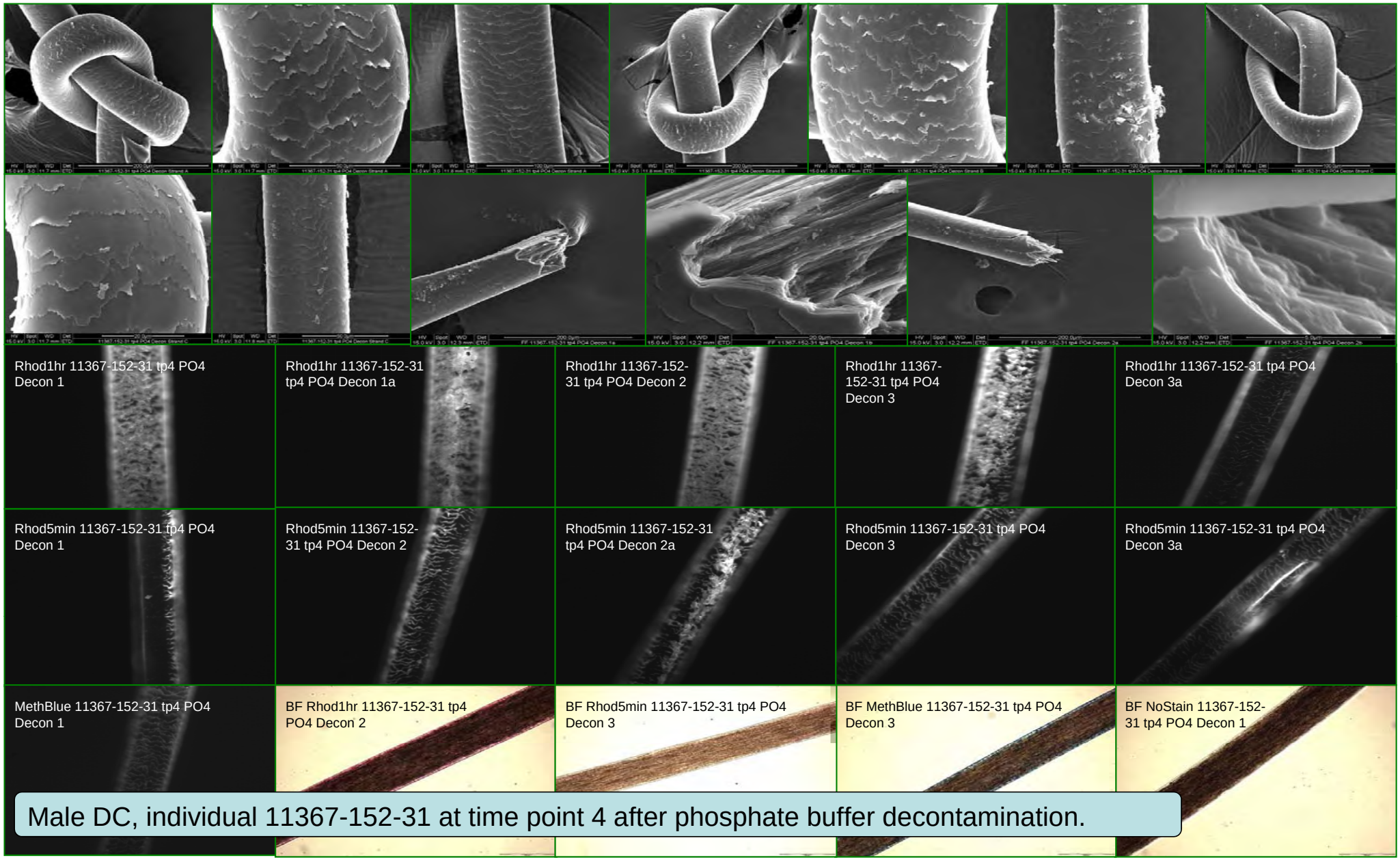

Rhod1hr 11367-152-25 tp4 PO4 Decon 1
Rhod1hr 11367-152-25 tp4 PO4 Decon 1a
Rhod1hr 11367-152-25 tp4 PO4 Decon 2
Rhod1hr 11367-152-25 tp4 PO4 Decon 3
Rhod1hr 11367-152-25 tp4 PO4 Decon 3a
Rhod1hr 11367-152-25 tp4 PO4 Decon 3b
Rhod5min 11367-152-25 tp4 PO4 Decon 1
Rhod5min 11367-152-25 tp4 PO4 Decon 1a
Rhod5min 11367-152-25 tp4 PO4 Decon 2
Rhod5min 11367-152-25 tp4 PO4 Decon 3
Rhod5min 11367-152-25 tp4 PO4 Decon 3a
MethBlue 11367-152-25 tp4 PO4 Decon 1
BF Rhod1hr 11367-152-25 tp4 PO4 Decon 2
BF Rhod5min 11367-152-25 tp4 PO4 Decon 3
BF MethBlue 11367-152-25 tp4 PO4 Decon 2
BF NoStain 11367-152-25 tp4 PO4 Decon 1
Female DC, individual 11367-152-25 at time point 4 after phosphate buffer decontamination.

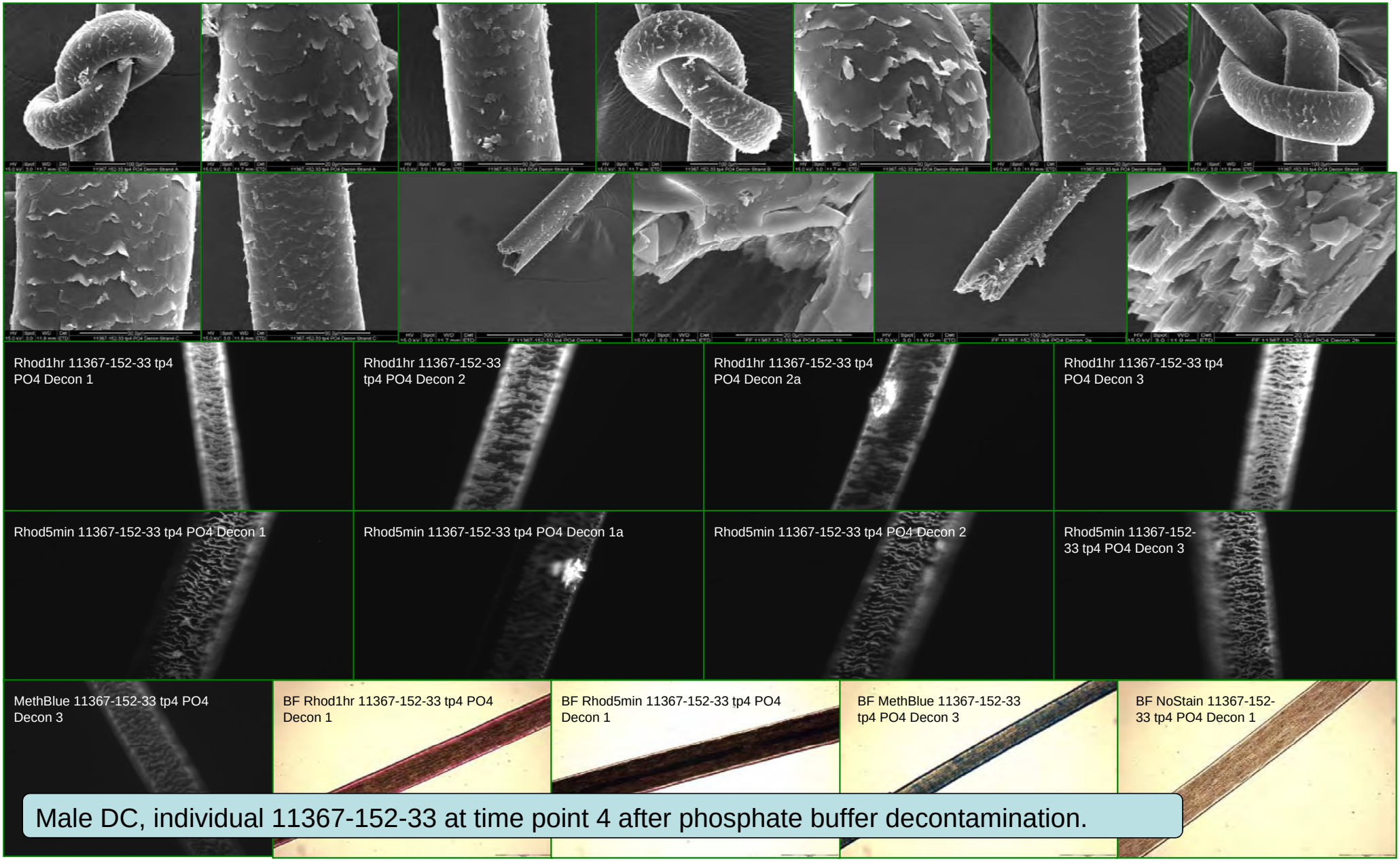

Rhod1hr 11367-152-26 tp4 PO4 Decon 1
Rhod1hr 11367-152-26 tp4 PO4 Decon 1a
Rhod1hr 11367-152-26 tp4 PO4 Decon 2
Rhod1hr 11367-152-26 tp4 PO4 Decon 2a
Rhod1hr 11367-152-26 tp4 PO4 Decon 3
Rhod1hr 11367-152-26 tp4 PO4 Decon 3a
Rhod5min 11367-152-26 tp4 PO4 Decon 1
Rhod5min 11367-152-26 tp4 PO4 Decon 1a
Rhod5min 11367-152-26 tp4 PO4 Decon 2
Rhod5min 11367-152-26 tp4 PO4 Decon 2a
Rhod5min 11367-152-26 tp4 PO4 Decon 3
MethBlue 11367-152-26 tp4 PO4 Decon 2
BF Rhod1hr 11367-152-26 tp4 PO4 Decon 3
BF Rhod5min 11367-152-26 tp4 PO4 Decon 1
BF MethBlue 11367-152-26 tp4 PO4 Decon 1
BF NoStain 11367-152-26 tp4 PO4 Decon 1
Female DC, individual 11367-152-26 at time point 4 after phosphate buffer decontamination.


Rhod1hr 11367-152-30 tp4 PO4 Decon 1
Rhod1hr 11367-152-30 tp4 PO4 Decon 1a
Rhod1hr 11367-152-30 tp4 PO4 Decon 2
Rhod1hr 11367-152-30 tp4 PO4 Decon 2a
Rhod1hr 11367-152-30 tp4 PO4 Decon 3
Rhod1hr 11367-152-30 tp4 PO4 Decon 3a
Rhod5min 11367-152-30 tp4 PO4 Decon 1
Rhod5min 11367-152-30 tp4 PO4 Decon 2
Rhod5min 11367-152-30 tp4 PO4 Decon 3
MethBlue 11367-152-30 tp4 PO4 Decon 1
BF Rhod1hr 11367-152-30 tp4 PO4 Decon 3
BF Rhod5min 11367-152-30 tp4 PO4 Decon 1
BF MethBlue 11367-152-30 tp4 PO4 Decon 2
BF NoStain 11367-152-30 tp4 PO4 Decon 1
Female DC, individual 11367-152-30 at time point 4 after phosphate buffer decontamination.

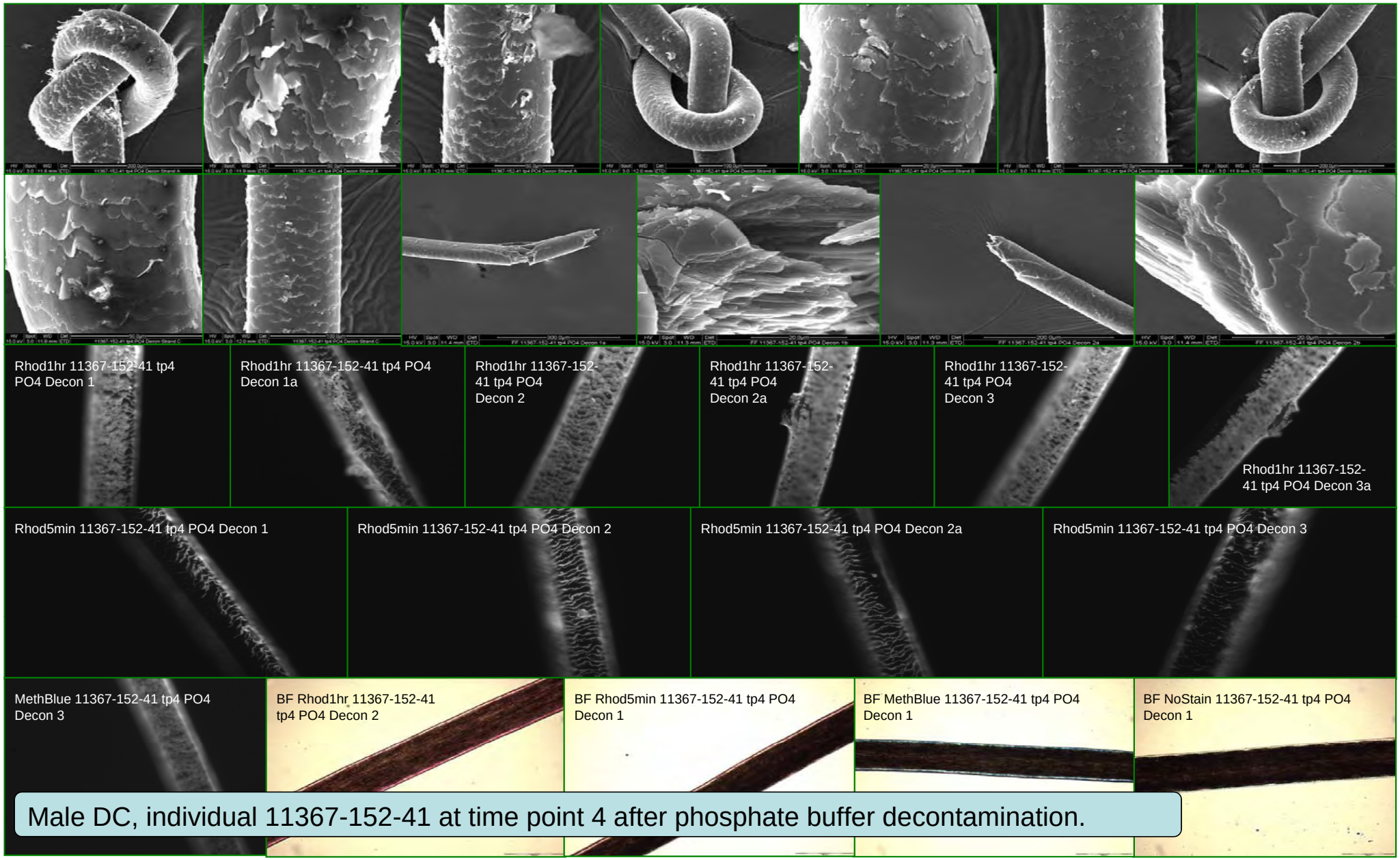

Rhod1hr 11367-152-46 tp4 PO4 Decon 1
Rhod1hr 11367-152-46 tp4 PO4 Decon 2
Rhod1hr 11367-152-46 tp4 PO4 Decon 3
Rhod5min 11367-152-46 tp4 PO4 Decon 1
Rhod5min 11367-152-46 tp4 PO4 Decon 2
Rhod5min 11367-152-46 tp4 PO4 Decon 2a
Rhod5min 11367-152-46 tp4 PO4 Decon 3
Rhod5min 11367-152-46 tp4 PO4 Decon 3a
MethBlue 11367-152-46 tp4 PO4 Decon 1
BF Rhod1hr 11367-152-46 tp4 PO4 Decon 3
BF Rhod5min 11367-152-46 tp4 PO4 Decon 1
BF MethBlue 11367-152-46 tp4 PO4 Decon 3
BF NoStain 11367-152-46 tp4 PO4 Decon 1
Male DC, individual 11367-152-46 at time point 4 after phosphate buffer decontamination.


Rhod1hr 11367-152-29 tp4 PO4 Decon 1
Rhod1hr 11367-152-29 tp4 PO4 Decon 1a
Rhod1hr 11367-152-29 tp4 PO4 Decon 2
Rhod1hr 11367-152-29 tp4 PO4 Decon 3
Rhod5min 11367-152-29 tp4 PO4 Decon 1
Rhod5min 11367-152-29 tp4 PO4 Decon 2
Rhod5min 11367-152-29 tp4 PO4 Decon 2a
Rhod5min 11367-152-29 tp4 PO4 Decon 3
MethBlue 11367-152-29 tp4 PO4 Decon 1
BF Rhod1hr 11367-152-29 tp4 PO4 Decon 3
BF Rhod5min 11367-152-29 tp4 PO4 Decon 1
BF MethBlue 11367-152-29 tp4 PO4 Decon 2
BF NoStain 11367-152-29 tp4 PO4 Decon 1
Male DC, individual 11367-152-29 at time point 4 after phosphate buffer decontamination.


Rhod1hr 11367-152-31 tp4 PO4 Decon 1
Rhod1hr 11367-152-31 tp4 PO4 Decon 1a
Rhod1hr 11367-152-31 tp4 PO4 Decon 2
Rhod1hr 11367-152-31 tp4 PO4 Decon 3
Rhod1hr 11367-152-31 tp4 PO4 Decon 3a
Rhod5min 11367-152-31 tp4 PO4 Decon 1
Rhod5min 11367-152-31 tp4 PO4 Decon 2
Rhod5min 11367-152-31 tp4 PO4 Decon 2a
Rhod5min 11367-152-31 tp4 PO4 Decon 3
Rhod5min 11367-152-31 tp4 PO4 Decon 3a
MethBlue 11367-152-31 tp4 PO4 Decon 1
BF Rhod1hr 11367-152-31 tp4 PO4 Decon 2
BF Rhod5min 11367-152-31 tp4 PO4 Decon 3
BF MethBlue 11367-152-31 tp4 PO4 Decon 3
BF NoStain 11367-152-31 tp4 PO4 Decon 1
Male DC, individual 11367-152-31 at time point 4 after phosphate buffer decontamination.



Male DC, individual 11367-152-33 at time point 4 after phosphate buffer decontamination.


Rhod1hr 11367-152-41 tp4 PO4 Decon 1
Rhod1hr 11367-152-41 tp4 PO4 Decon 1a
Rhod1hr 11367-152-41 tp4 PO4 Decon 2
Rhod1hr 11367-152-41 tp4 PO4 Decon 2a
Rhod1hr 11367-152-41 tp4 PO4 Decon 3
Rhod1hr 11367-152-41 tp4 PO4 Decon 3a
Rhod5min 11367-152-41 tp4 PO4 Decon 1
Rhod5min 11367-152-41 tp4 PO4 Decon 2
Rhod5min 11367-152-41 tp4 PO4 Decon 2a
Rhod5min 11367-152-41 tp4 PO4 Decon 3
MethBlue 11367-152-41 tp4 PO4 Decon 3
BF Rhod1hr 11367-152-41 tp4 PO4 Decon 2
BF Rhod5min 11367-152-41 tp4 PO4 Decon 1
BF MethBlue 11367-152-41 tp4 PO4 Decon 1
BF NoStain 11367-152-41 tp4 PO4 Decon 1
Male DC, individual 11367-152-41 at time point 4 after phosphate buffer decontamination.