AMA Analytical Services, Inc.
Focused On Results.

CERTIFICATE OF ANALYSIS

Chain of Custody: 305281
Client: US Food & Drug Administration
Address: Office of Cosmetics & Colors
4300 River Road
College Park, MD 20740
Attention: John Gasper

Job Name: Task 2 - Analysis of Demonstration Samples
Job Location: Talc (x000756) A TK4428 FDA
Job Number: Not Provided
PO Number: HHSF223201810337P

Date Submitted: 11/14/2018
Date Analyzed: 1/31/2019
Report Date: 2/21/2019
Date Sampled: Not Provided
Person Submitting: John Gasper
Revised: 1/23/2020 (Revision #4)

Summary of Analysis

AMA Sample ID	Client Sample ID	TEM LOD Using ASTM D5756 Mass Calculation	TEM LOQ Using ASTM D5756 Mass Calculation	% Tremolite by TEM Using ASTM D5756 Mass Calculation	% Chrysotile by TEM Using ASTM D5756 Mass Calculation	% Total Tremolite and Chrysotile by TEM Using ASTM D5756 Mass Calculation	% Asbestos by PLM	% Organics	% Acid Soluable	% Other	Comments
(b) (6)											
305281-4	D-30	0.00001074%	0.00004295%	15.49345%	ND	15.49345%	ND	9.4%	46.0%	29.0%	
305281-4A	D-30	0.00000519%	0.00002076%	1.82317%	ND	1.82317%	<0.13%	8.4%	41.2%	48.6%	
305281-4B	D-30	0.00000712%	0.00002848%	4.32456%	ND	4.32456%	ND	8.9%	41.7%	45.2%	
305281-5	D-31	0.00000161%	0.00000644%	0.24245%	0.00183%	0.24429%	<0.18%	13.4%	14.7%	71.7%	
305281-5A	D-31	0.00000229%	0.00000916%	0.26810%	0.00035%	0.26845%	<0.18%	13.7%	13.5%	72.5%	
305281-5B	D-31	0.00000117%	0.00000468%	0.20999%	0.00012%	0.21011%	<0.18%	13.5%	13.7%	72.6%	
(b) (6)											

LOD = Limit of Detection **LOQ** = Limit of Quantification **ND** = Not Detected **PLM** = Polarized Light Microscopy **TEM** = Transmission Electron Microscopy

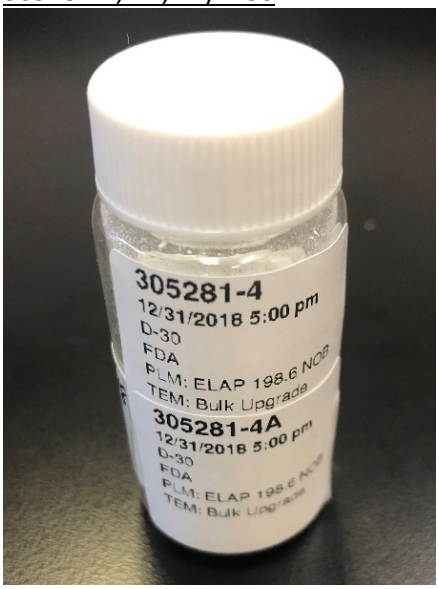
Analytical Method(s): PLM by Modified NY ELAP 198.6
TEM by Modified NY ELAP 198.4/ASTM D5756

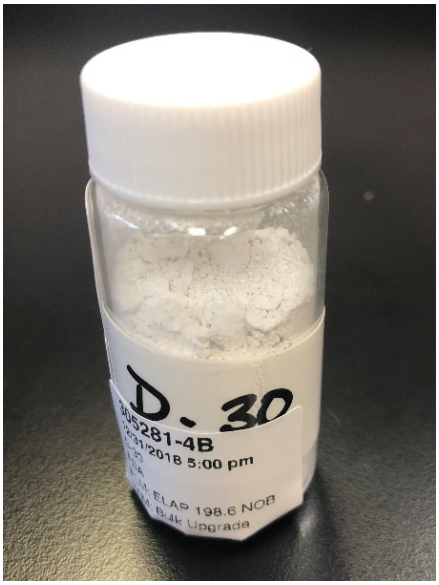
Analyst(s): **PLM** (b) (6)
TEM (b) (6)

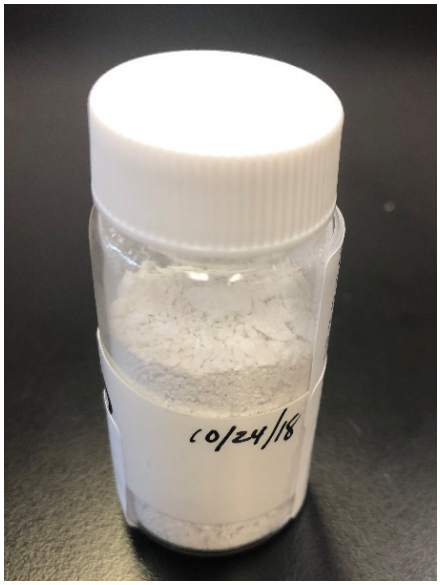
Technical Director: Andreas Saldivar

All results are to be considered preliminary and subject to change unless signed by the Technical Director or Deputy

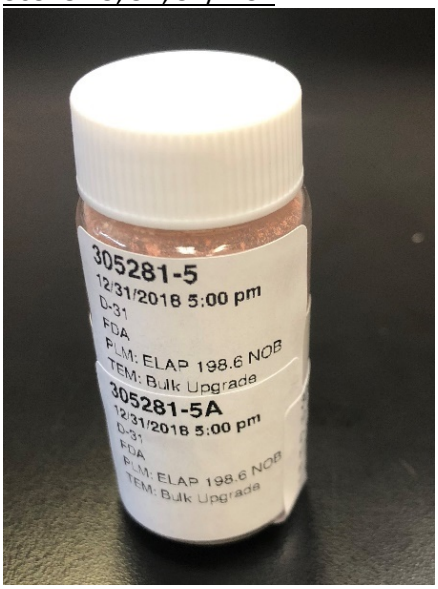
305281-4, 4A, 4B/D-30

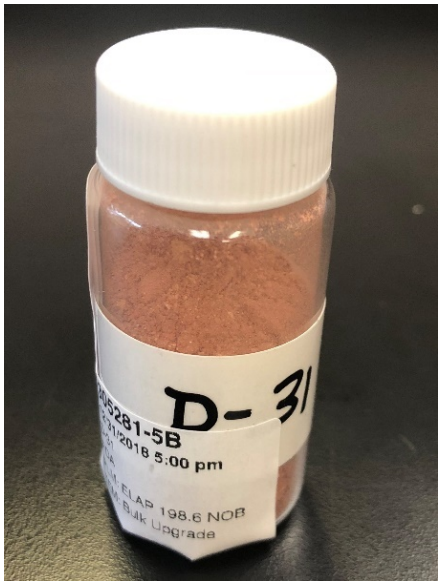




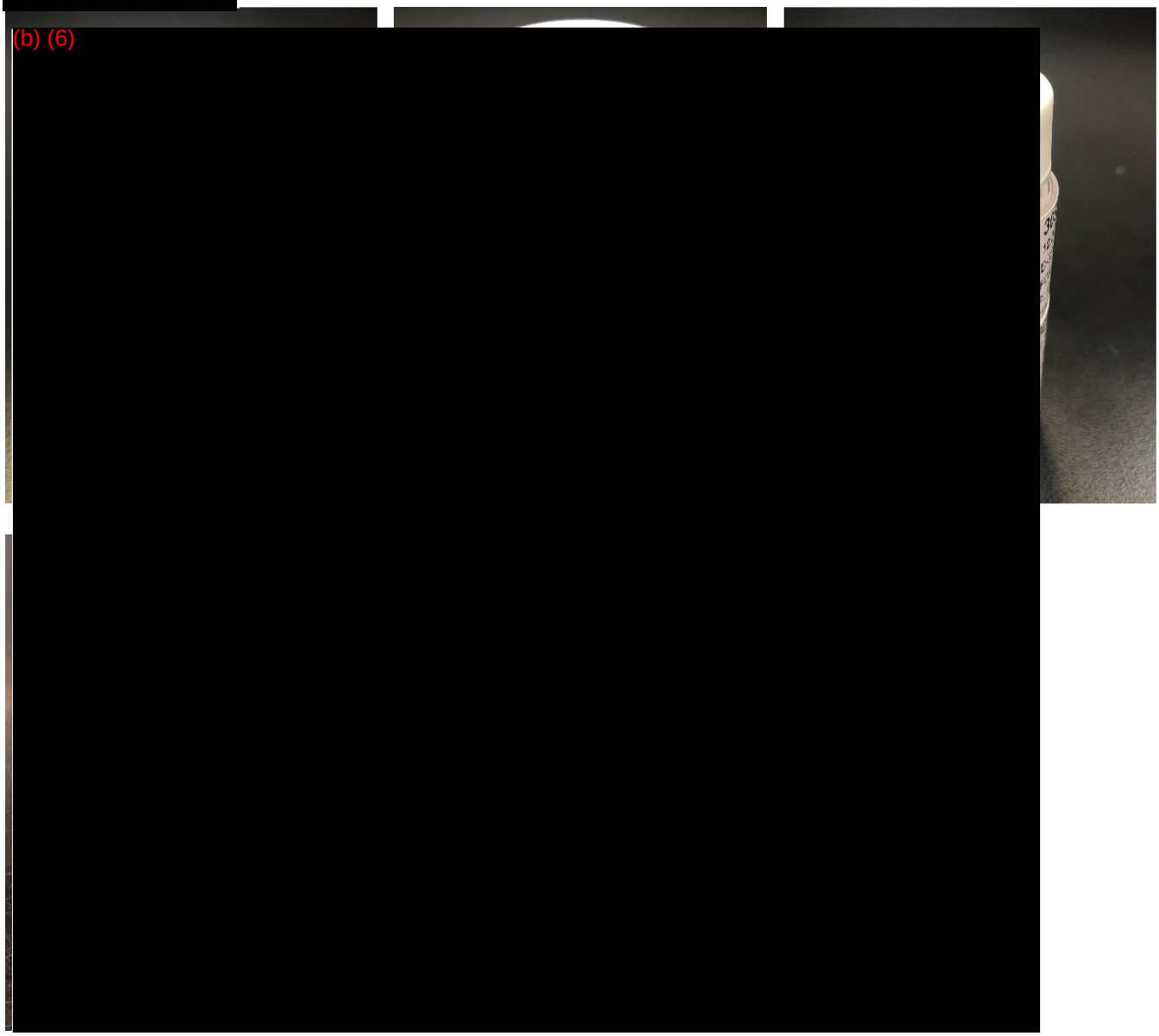


305281-5, 5A, 5B/D-31





(b) (6)



Sample Preparation for Samples 305281-3 through 305281-6:
Samples were prepared for PLM and TEM bulk analysis by (b) (6) on December 27, 2018 through January 7, 2019. Sample preparation consisted of the following steps:

1) Label and weigh a single 8mL glass vial for each sample in the set.
2) Weigh out 0.5 to 0.8 grams of material and place in corresponding 8mL glass vial. Record weight.
3) Burn samples at 480° C for at least 12 hours.
4) Record Post-Ash Weight.
5) Treat ashed sample with concentrated hydrochloric acid.
6) Filter acid washed material onto a pre-weighed 47mm 0.4um PolyCarbonate filter.
7) Place filter into drying oven for 30 minutes and then record Post-Acid Wash weight.
8) Place filter into corresponding 100ml jar.
9) Make four slide preparations for each sample from the filter residue in 1.550 dispersion oil for PLM analysis. Make additional preparations in 1.605, 1.625, 1.680 and/or 1.700 dispersion oil if necessary.
10) Weigh a portion of the residue and place it in a 2nd 100ml jar.
11) Fill the 100ml jar with deionized water
12) Sonicate the jar for approximate 5-minutes.

13) Filter 0.2ml to 1ml of the solution onto a 47mm 0.22um MCE filter.
14) Dry the filter for 10 minutes then collapse, carbon coat, and place on a 3 TEM grids.

PLM Analysis for Samples 305281-3 through 305281-6:
Analysis was performed in accordance with NY ELAP 198.6 protocols. The analysis was conducted using an Olympus BH-2 polarized light microscope (PLM) equipped with a dispersion staining objective. All four slide preparations for each aliquot were examined. 400-point count was performed for those samples on which asbestos was observed. If no asbestos was detected on any of the slides, the percentage of fibrous components were determined by visual estimation. The results of this analysis are detailed below in the *Discussion and Interpretation of Analytical Findings* section for each individual sample.

TEM Analysis for Samples 305281-3 through 305281-6:
Analysis was performed in accordance with modified NY ELAP Method 198.4 protocols. The analysis was performed using a JEOL JEM-100CX II transmission electron microscope (TEM), equipped with a Thermo Fisher Quest Energy Dispersive X-Ray Analyzer (EDXA), at magnifications of 19,000x. Two grids for each aliquot were examined. 20 grid openings were examined per sample.

Modifications to the NY ELAP 198.4 Method were:

1) The residue was not placed in alcohol and prepared using the quick drop method. To obtain a more uniform preparation, the residue was placed in a jar and filled with 100ml of deionized water. The jar was sonicated and a portion of the solution was filtered onto a 47mm 0.22um MCE filter.
2) The tremolite and chrysotile were not visually estimated. The length and width of the observed particles were measured and the mass of each amphibole particle was calculated using the ASTM D5756 and ISO 22262-2 methods. For chrysotile the ASTM D5756 equation was used to calculate the mass. All particles identified as asbestos were included with the counts/concentrations, regardless of size or whether or not they would be considered "asbestiform."

The results of this analysis are detailed below in the *Discussion and Interpretation of Analytical Findings* section for each individual sample.

Calculations

ASTM D5756 Mass

$M = \pi/4\ L * W^2 * D * 10^{-12}$

ISO 22262-2

$M(g) = L * W * \frac{1}{2}W * D * 10^{-12}$

M = mass
L = length
W = width
D = density

Percent Calculation

$$\frac{EFA(mm^2) * 100ml * MA(g) * RW(g)}{VF(ml) * IW(g) * AA(mm^2) * RJ(g)}$$

The calculated value is then multiplied by 100 to convert it to percent.

EFA – Effective filter area
MA – Mass of asbestos
RW – Weight of residue
VF – Volume filtered
IW – Initial weight of the sample
AA – Area analyzed

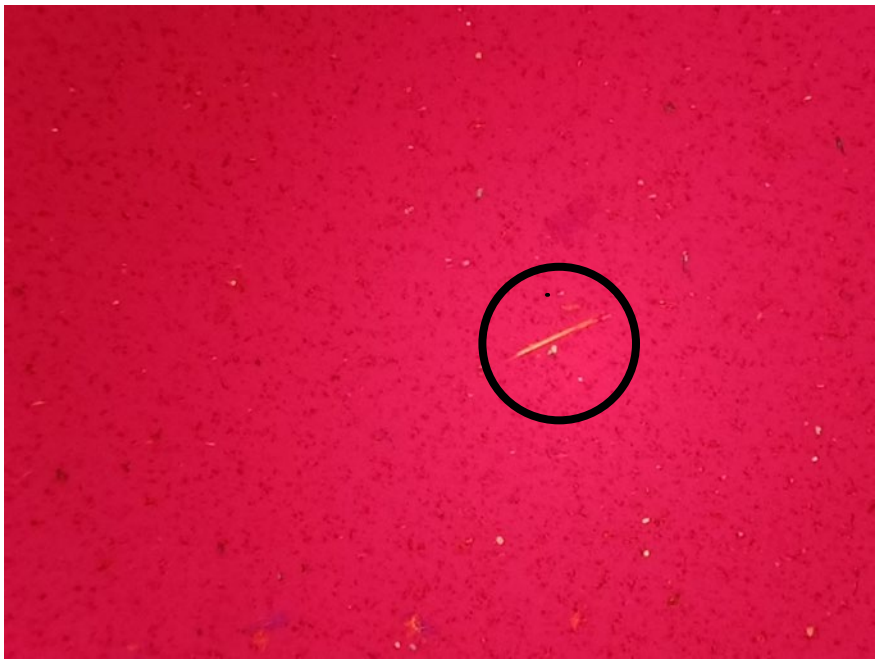
305281-4, 4A, 4B, Client Sample D30

PLM

All three aliquots of sample D30 were analyzed by (b) (6) on January 31, 2019. Tremolite was observed on aliquot 4a but no points were counted. No tremolite was detected on 4 and 4b. No other asbestos was detected.

305281-4	NAD
305281-4A	<0.13% Tremolite detected
305281-4B	NAD

305281-4A Tremolite 100x



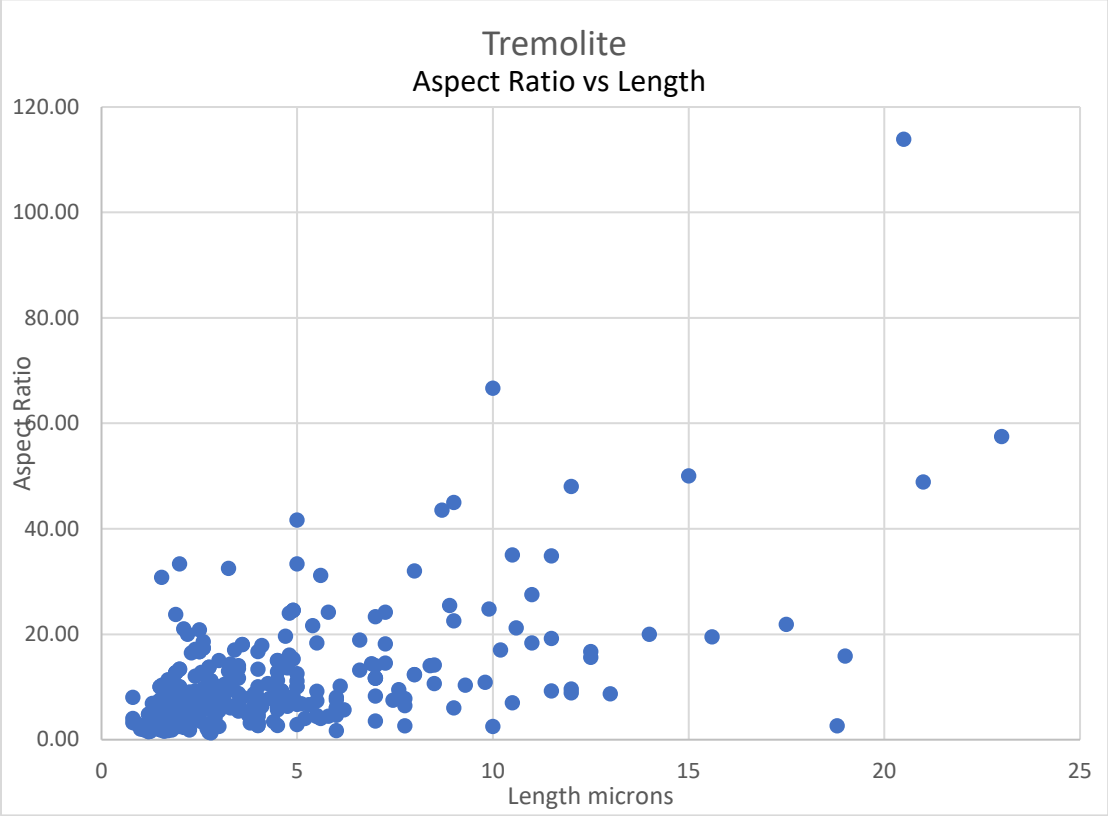
TEM

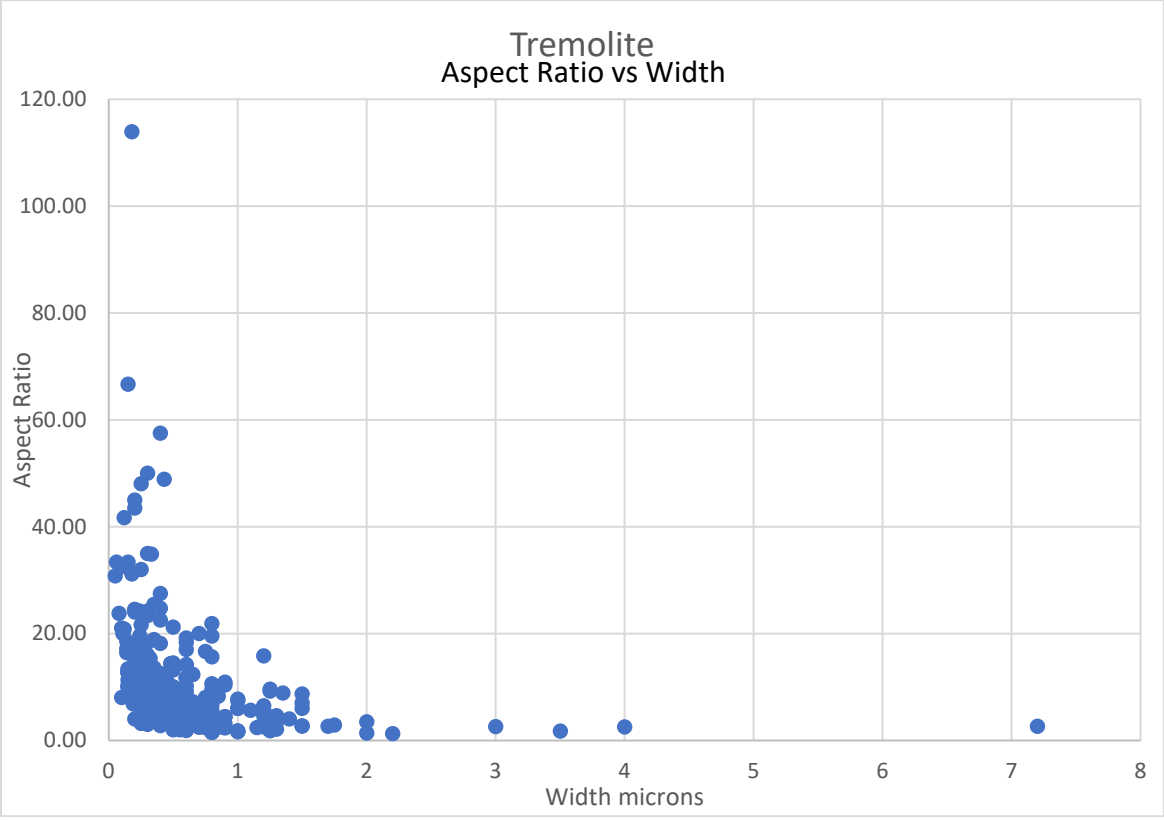
Sample 4 was analyzed by (b) (6) on January 11, 2019. Samples 4A and 4B were analyzed by (b) (6) on January 25, 2019 and January 31, 2019 respectively. Talc is the main constituent in all three aliquots. The talc is primarily in plate form but there are also some talc fibers and ribbons. Tremolite was detected on all three aliquots. At least 100 tremolite particles were documented for each aliquot. The length and width of each particle was recorded and the mass was calculated using both the ASTM D5756 and ISO 22262-2 methods. The percentage of tremolite was calculated using the mass from both calculations and the range was reported.

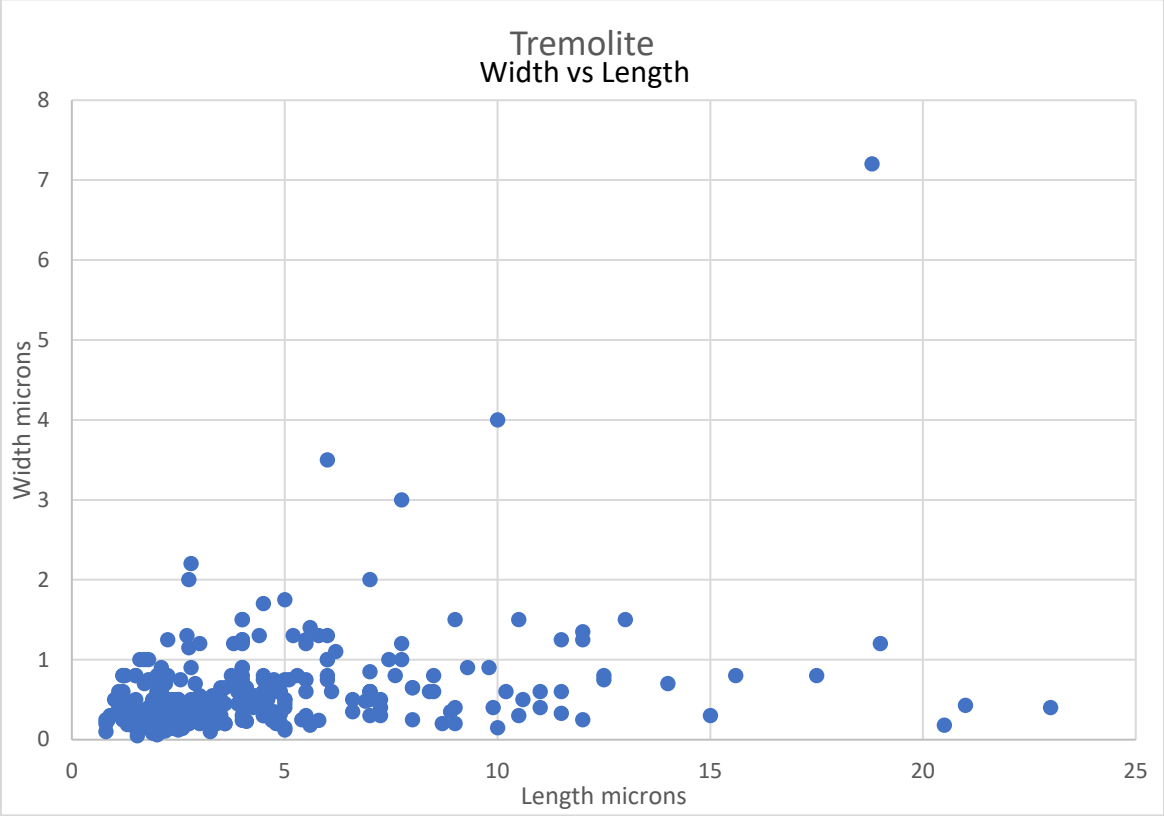
305281-4	9.86% to 15.5%
305281-4a	1.35% to 1.82%
305281-4b	2.75% to 4.32%

The range of tremolite in aliquot 4 is substantially higher than 4a and 4b. One large particle, #24, accounts for 85% of the total mass of tremolite counted in aliquot 4. When the mass of that particle is removed from the total the range of tremolite is 1.54% - 2.41%. The dramatic effect of this one particle on the total percent is a result of the dilution factors from preparation. The tremolite particles varied in morphology from small blocks to very long, high length to width aspect ratio fibers. The length to width aspect ratio of particles counted ranged from 1.27 to 113.9. The mean aspect ratio is 11.5. The chemistry of the tremolite observed consisted of O, Mg, Si, and Ca. A few particles had small amounts of Al and Fe. One chrysotile fiber was counted on sample 305281-4a.

The following chart is a plot aspect ratio vs length for all the particles counted over all three aliquots.

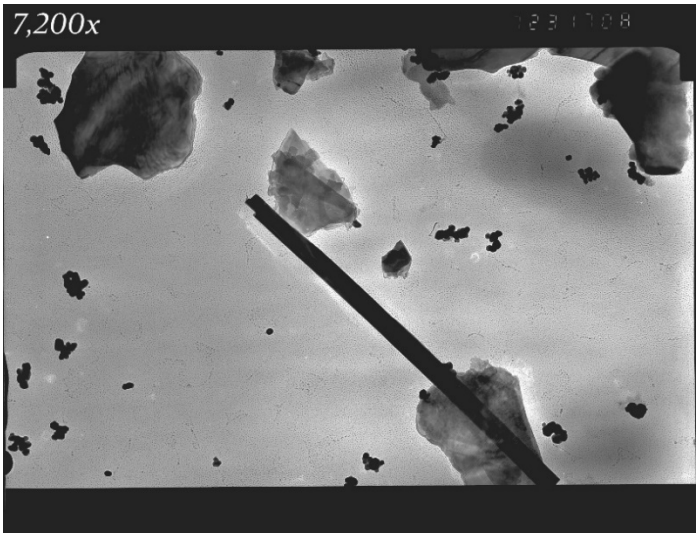

Tremolite
Aspect Ratio vs Length
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Aspect Ratio
0
5
10
15
20
25
Length microns

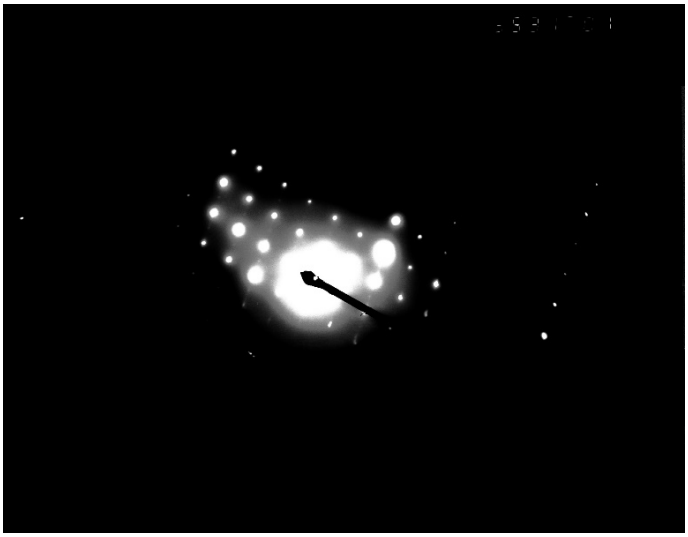

Tremolite
Aspect Ratio vs Width
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Aspect Ratio
0
1
2
3
4
5
6
7
8
Width microns

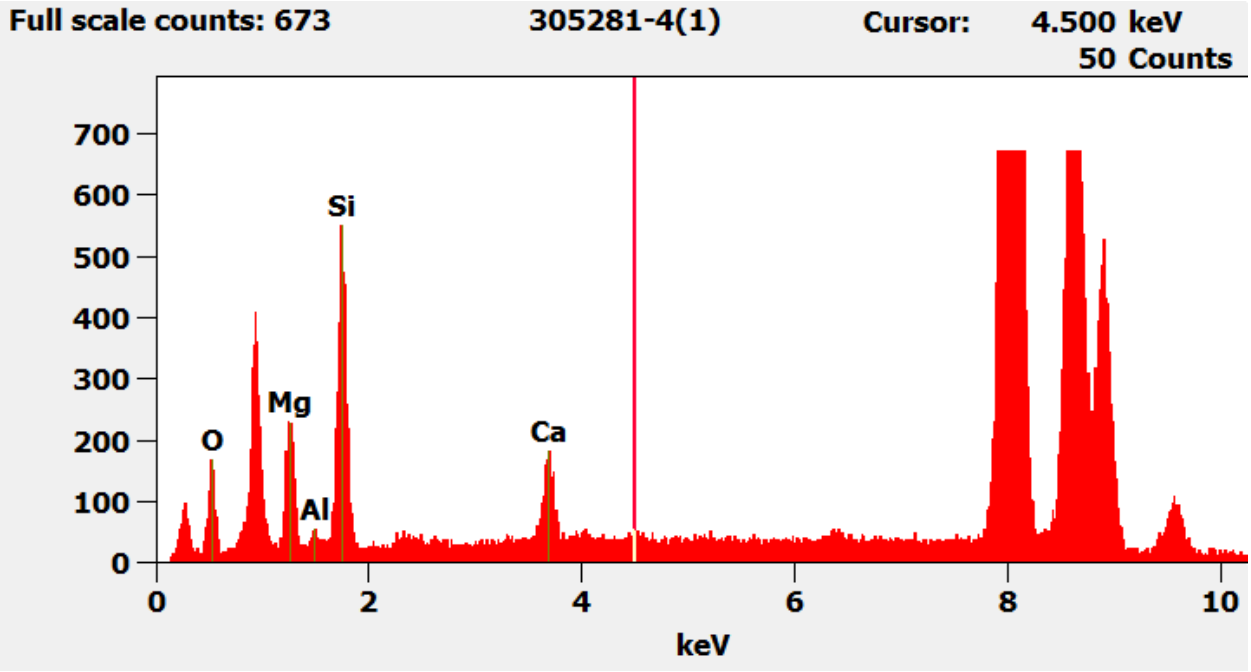


Below are pictures, diffraction patterns, and chemistry from some of the counted particles. The unidentified peaks in chemistry spectra are copper, zinc, and carbon. Those peaks are from the TEM specimen holder and specimen grid.

305281-4 Particle 2 8.9 x 0.35 microns Tremolite

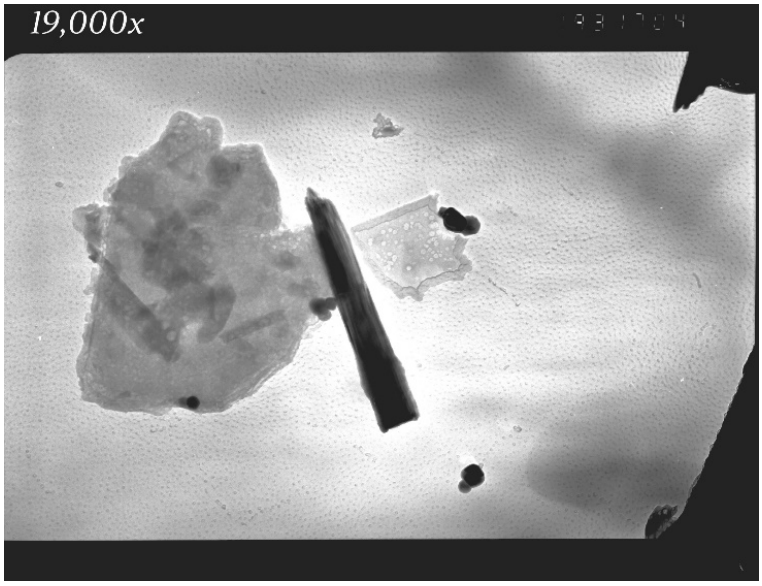


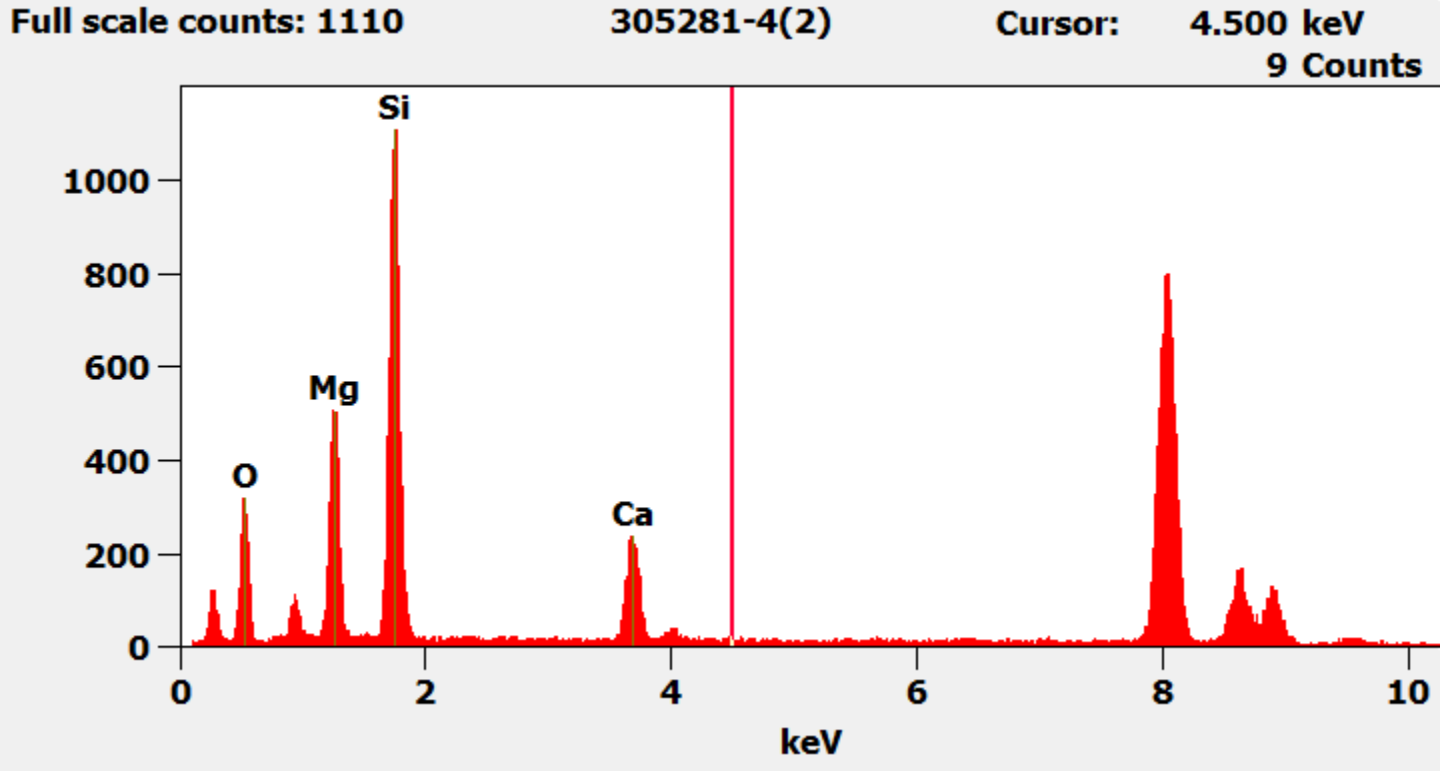




Element Line	Weight %	Weight % Error
O K	51.41	± 2.37
Mg K	18.09	± 0.54
Al K	1.98	± 0.29
Si K	25.57	± 0.35
Si L	---	---
Ca K	2.96	± 0.19
Ca L	---	---
Total	100.00	

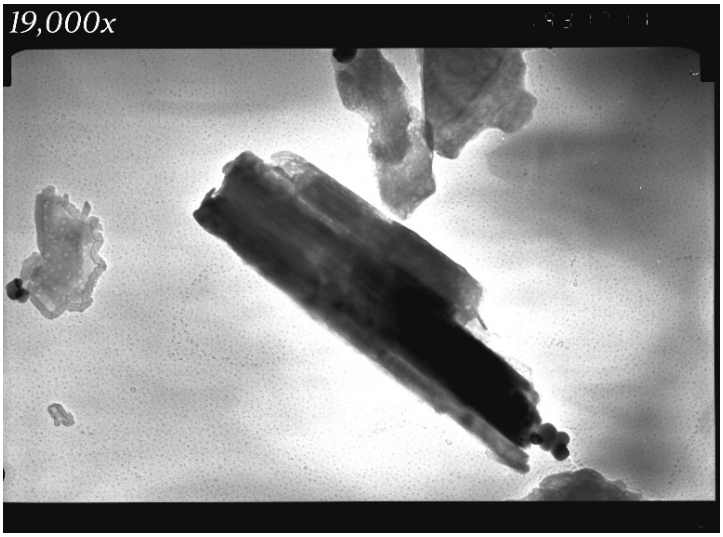
305281-4 Particle 3 1.6 x 0.2 microns Tremolite

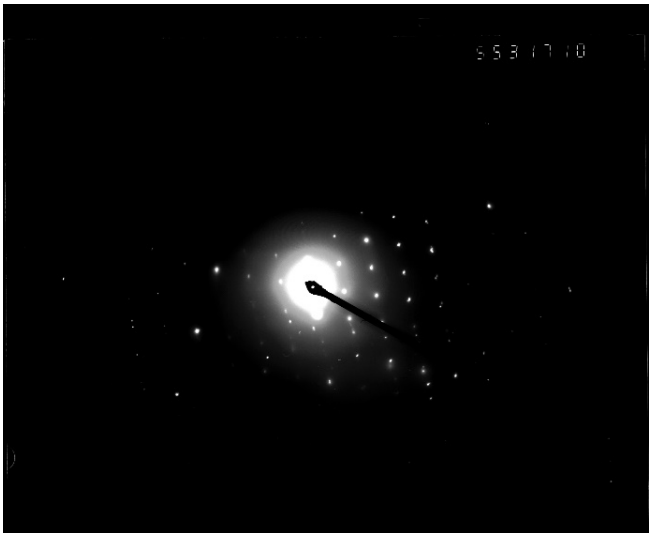


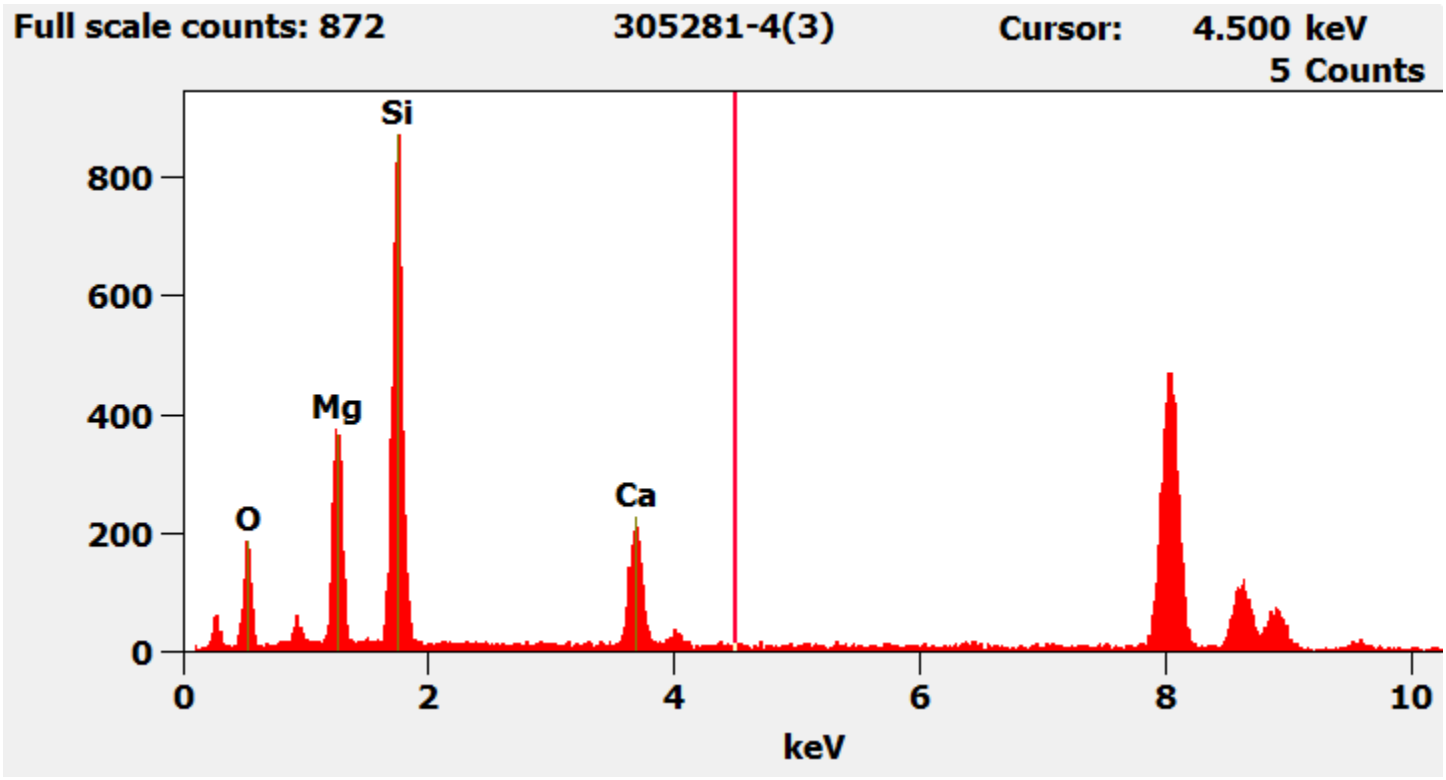


Element Line	Weight %	Weight % Error
O K	53.11	± 1.38
Mg K	16.92	± 0.30
Si K	27.08	± 0.30
Si L	---	---
Ca K	2.89	± 0.09
Ca L	---	---
Total	100.00	

305281-4 Particle 4 3.0 x 1.2 microns Tremolite

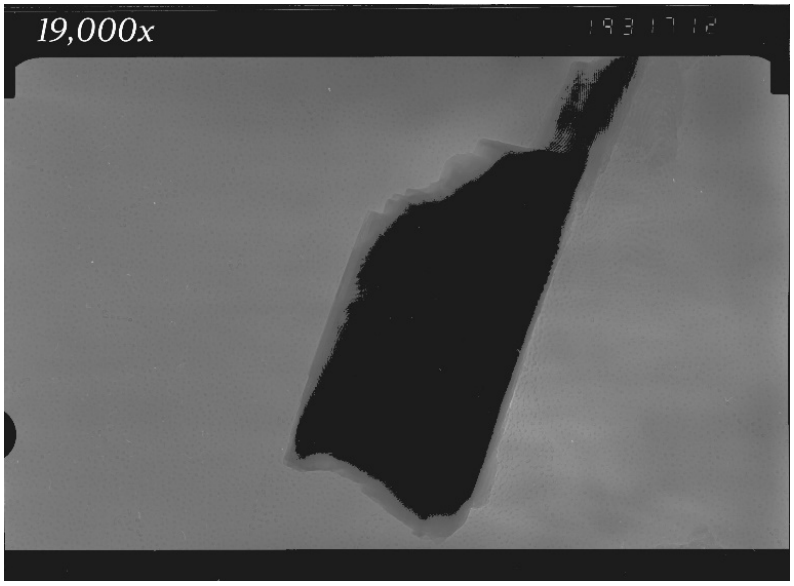


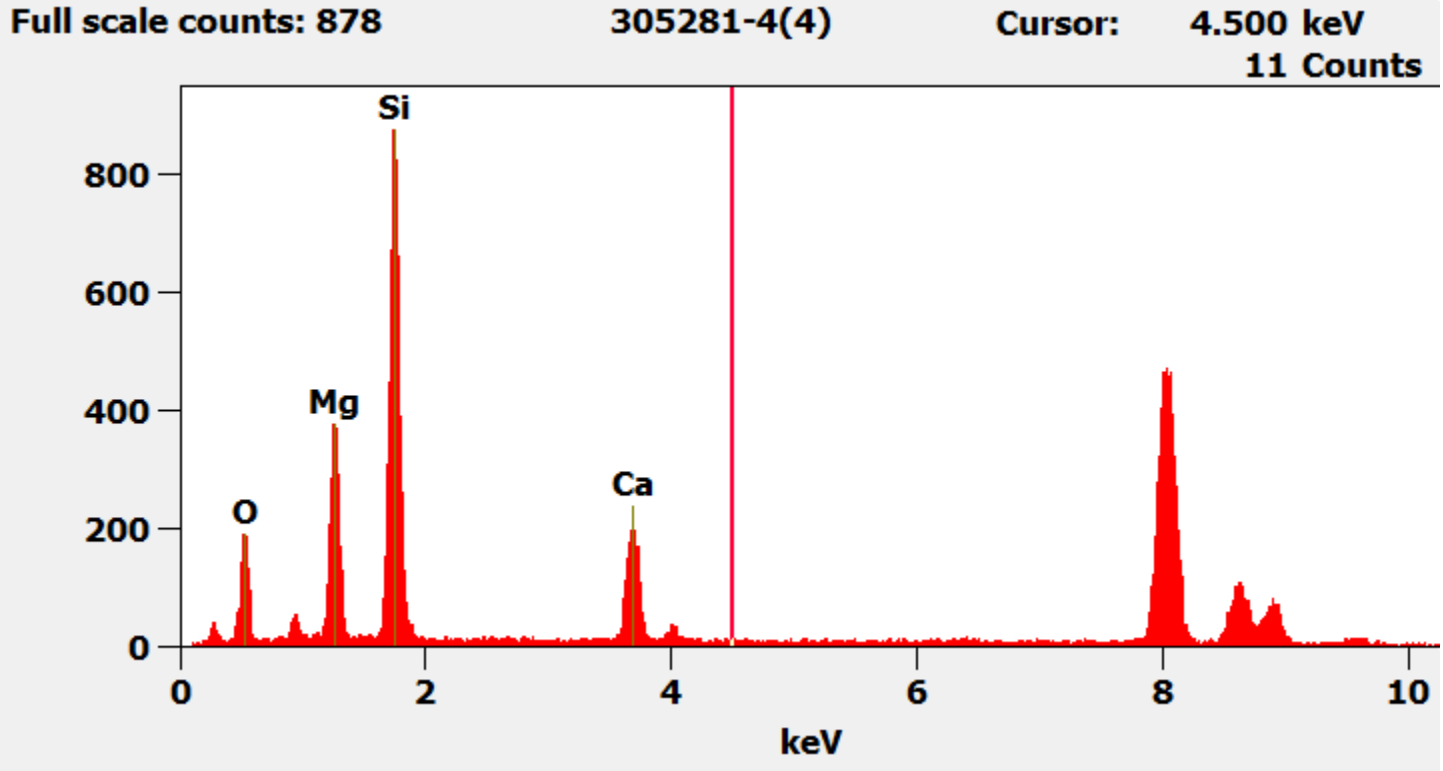




Element Line	Weight %	Weight % Error
O K	48.04	± 1.69
Mg K	17.67	± 0.35
Si K	30.30	± 0.38
Si L	---	---
Ca K	3.99	± 0.13
Ca L	---	---
Total	100.00	

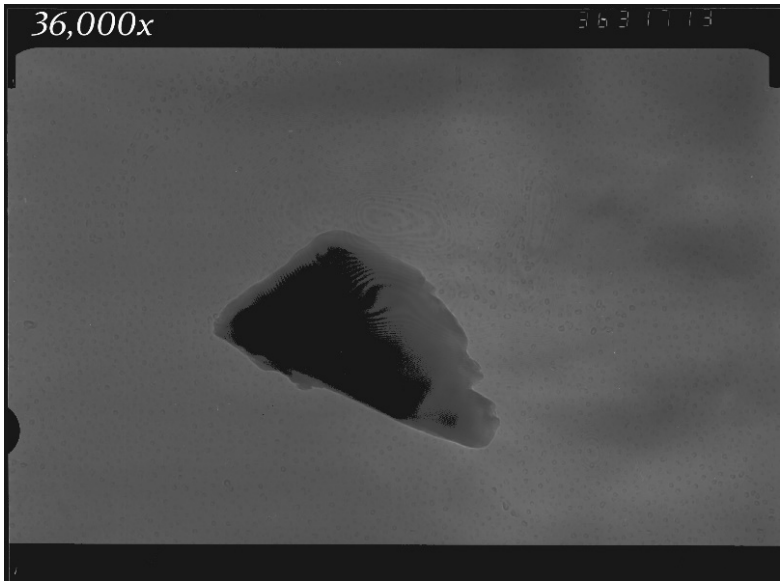
305281-4 Particle 5 2.7 x 1.3 microns Tremolite

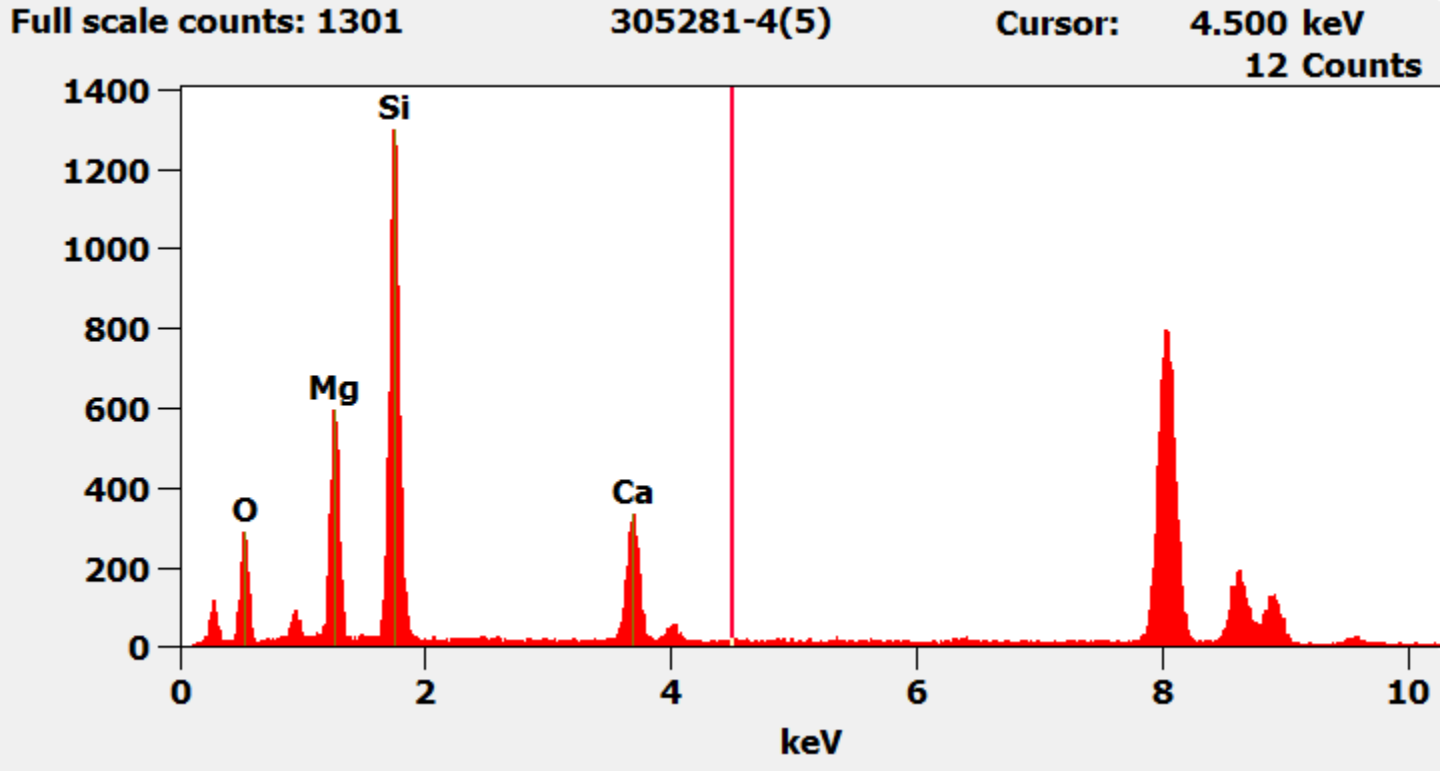




Element Line	Weight %	Weight % Error
O K	50.90	± 1.56
Mg K	16.75	± 0.34
Si K	28.70	± 0.35
Si L	---	---
Ca K	3.65	± 0.12
Ca L	---	---
Total	100.00	

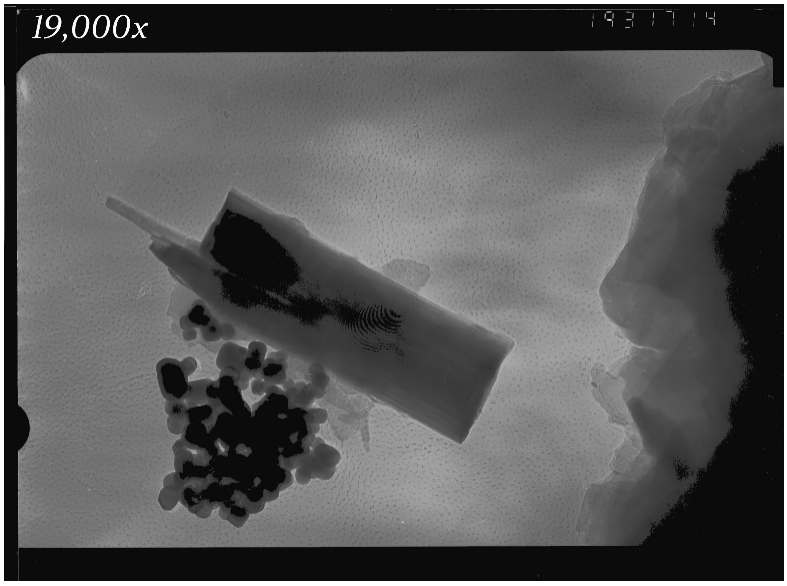
305281-4 Particle 6 1.2 x 0.8 microns Tremolite

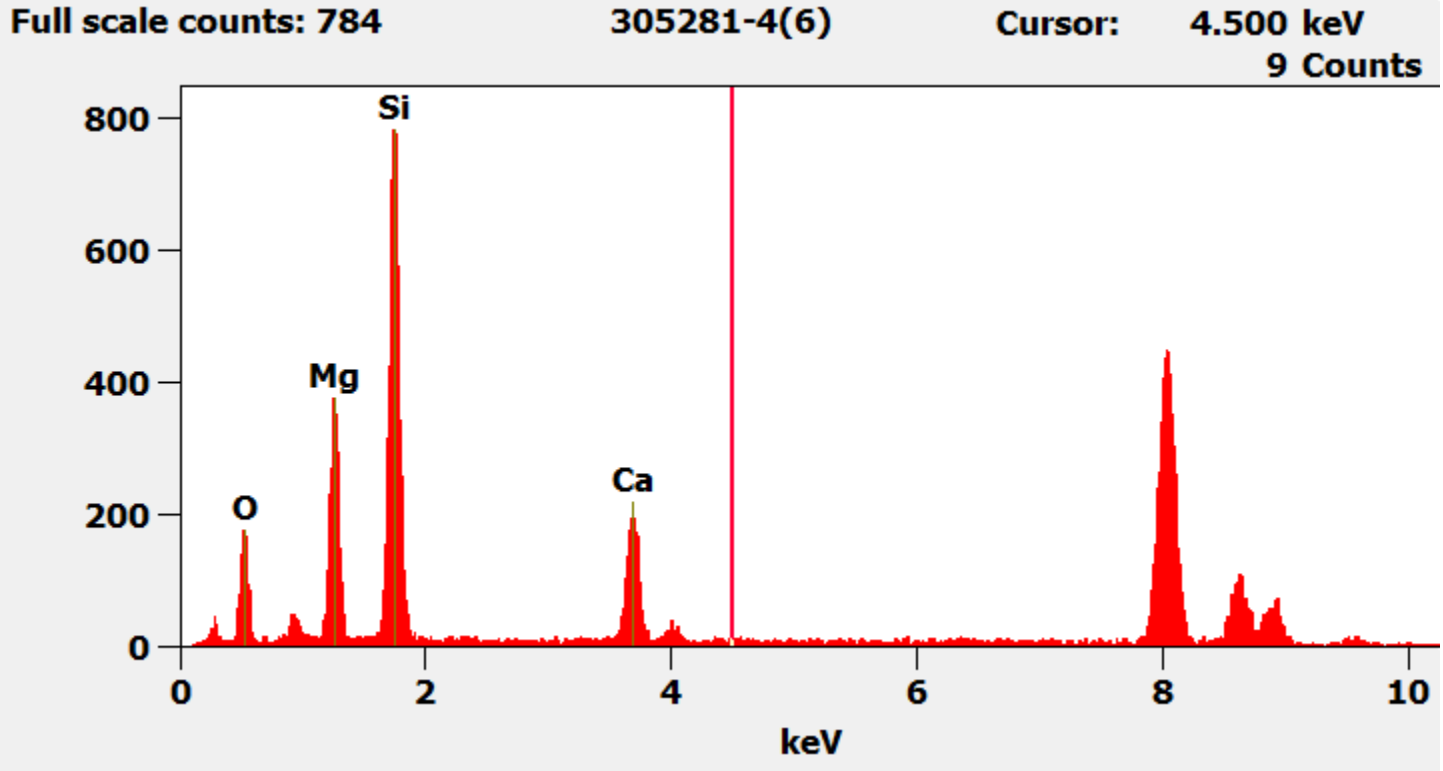




Element Line	Weight %	Weight % Error
O K	49.72	± 1.38
Mg K	17.25	± 0.29
Si K	29.30	± 0.30
Si L	---	---
Ca K	3.74	± 0.10
Ca L	---	---
Total	100.00	

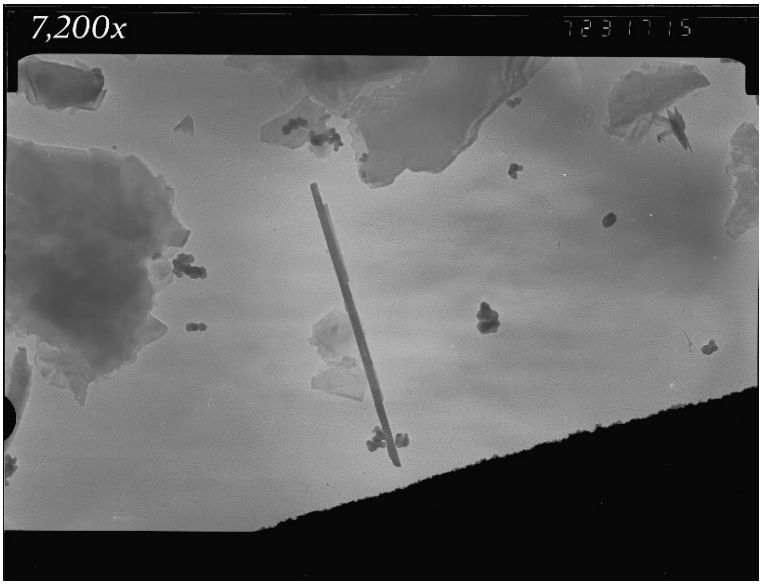
305281-4 Particle 7 2.3 x .8 microns Tremolite

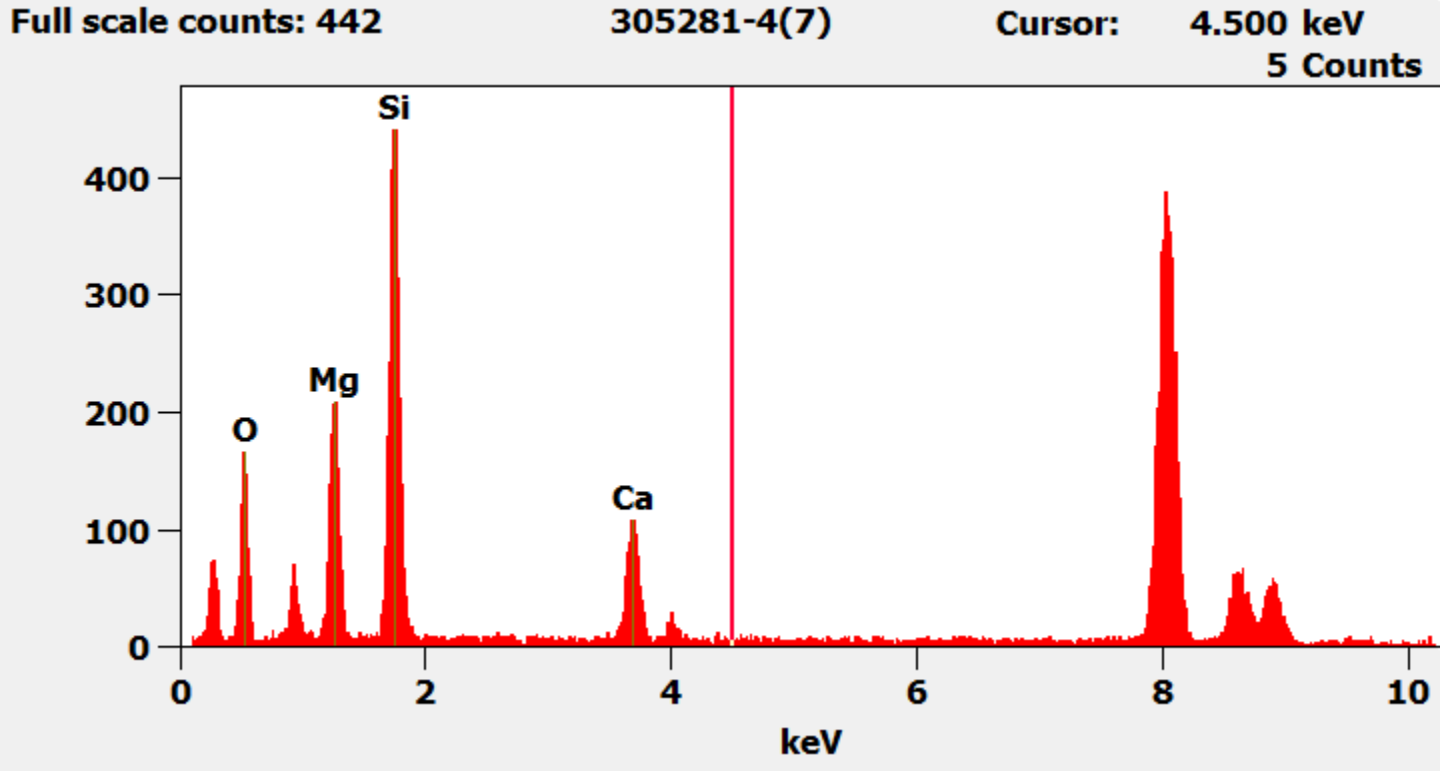




Element Line	Weight %	Weight % Error
O K	49.62	± 1.60
Mg K	17.26	± 0.35
Si K	29.41	± 0.37
Si L	---	---
Ca K	3.71	± 0.12
Ca L	---	---
Total	100.00	

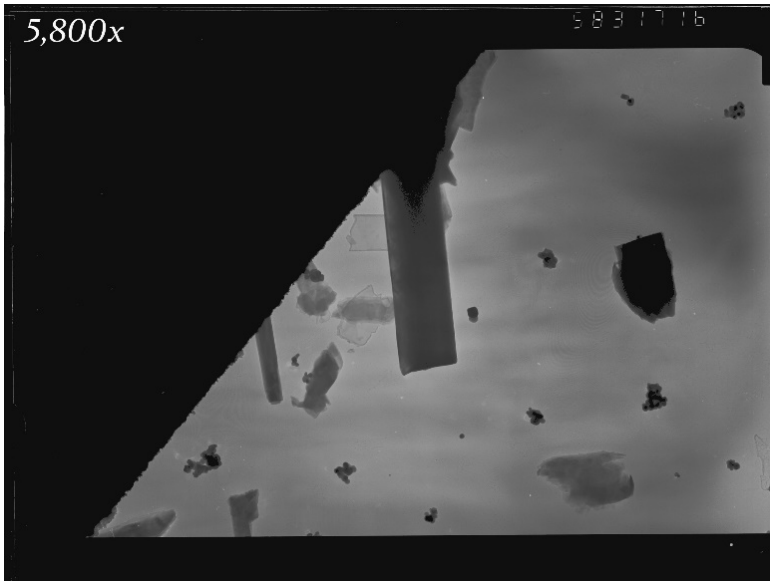
305281-4 Particle 8 5.6 x 0.18 microns Tremolite

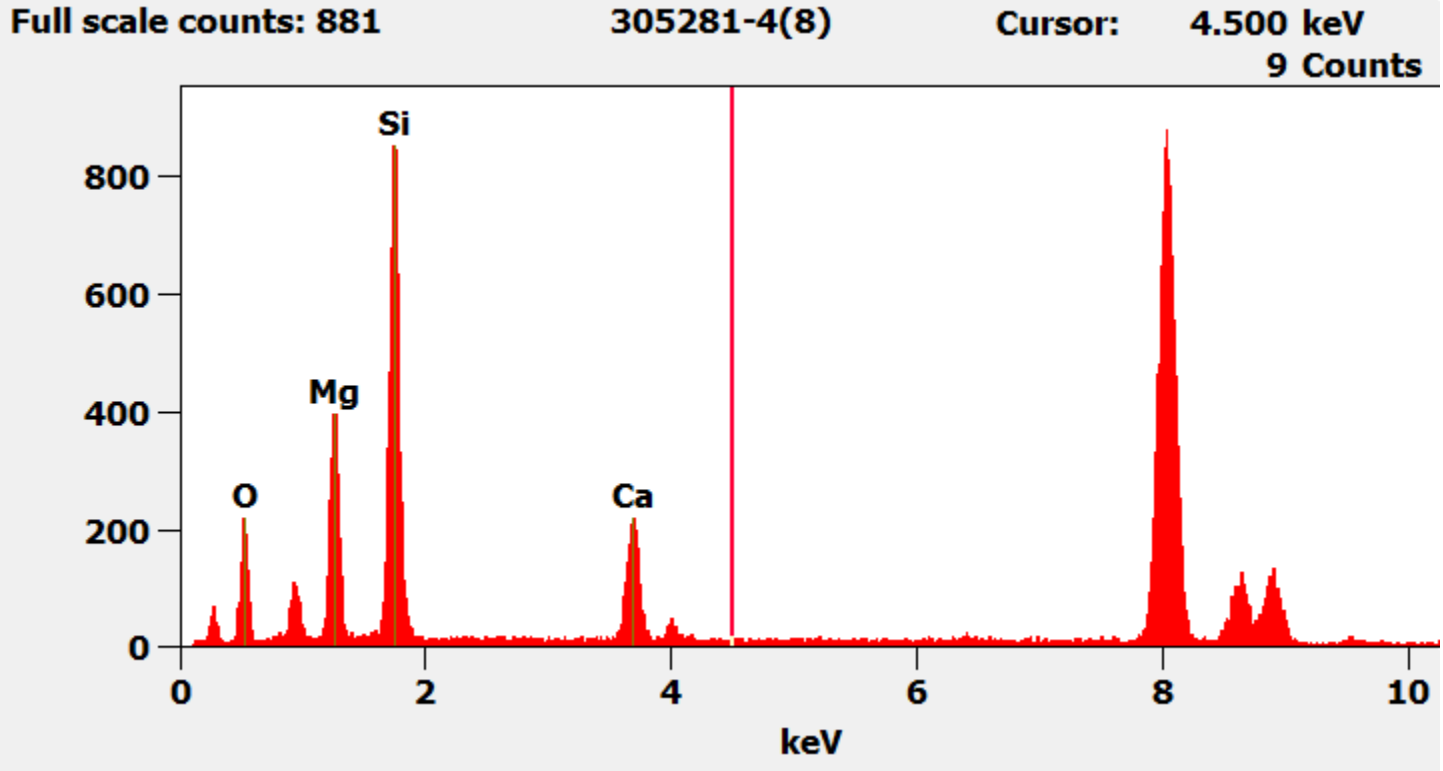




Element Line	Weight %	Weight % Error
O K	55.70	± 2.05
Mg K	16.34	± 0.44
Si K	25.17	± 0.42
Si L	---	---
Ca K	2.79	± 0.13
Ca L	---	---
Total	100.00	

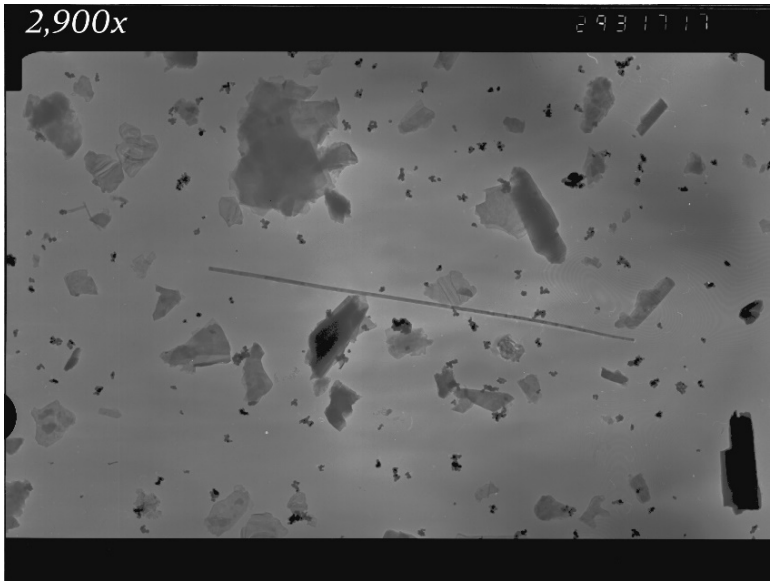
305281-4 Particle 9 5.8 x 1.3 microns Tremolite (#9 is the large particle. Particle 10 is also pictured to the left of 9)

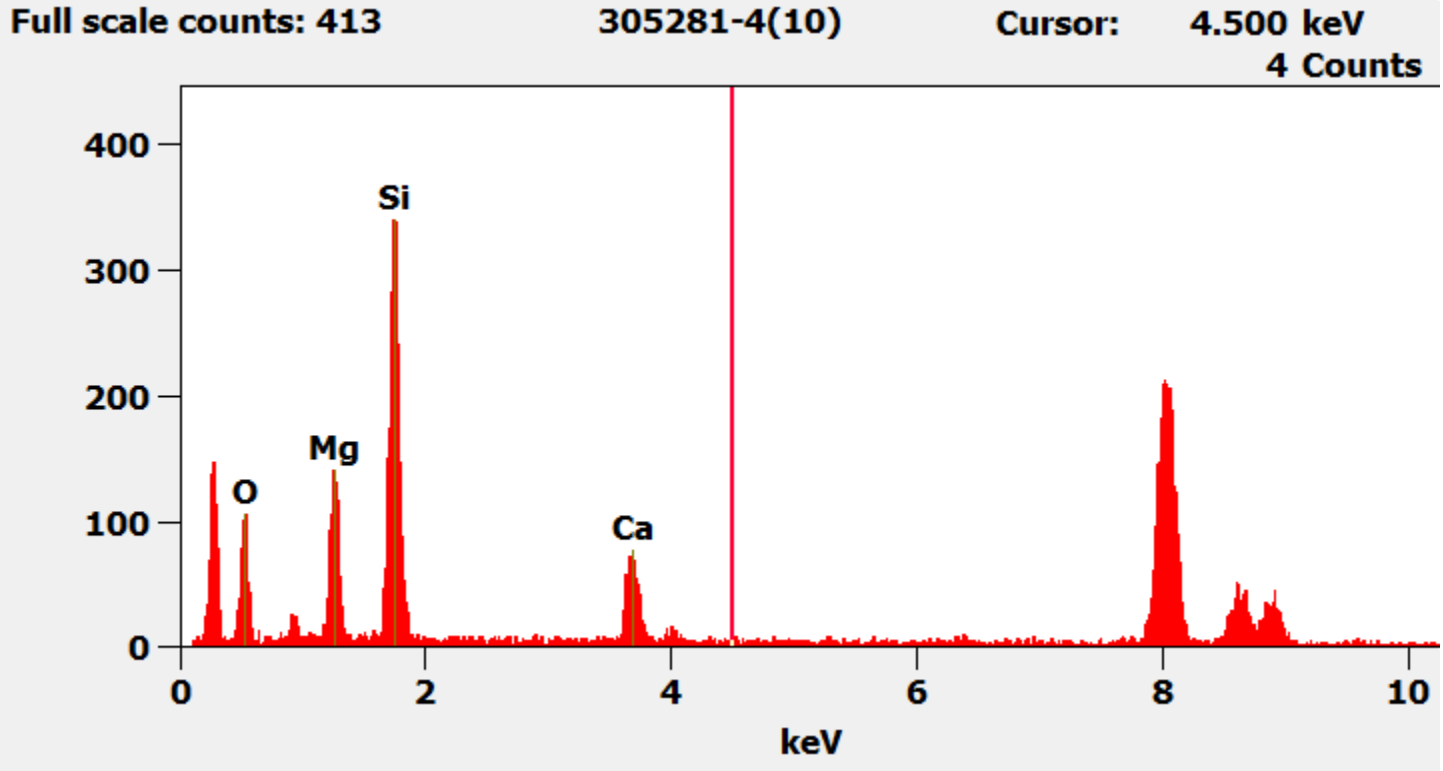




Element Line	Weight %	Weight % Error
O K	50.52	± 1.60
Mg K	18.00	± 0.35
Si K	27.86	± 0.35
Si L	---	---
Ca K	3.63	± 0.12
Ca L	---	---
Total	100.00	

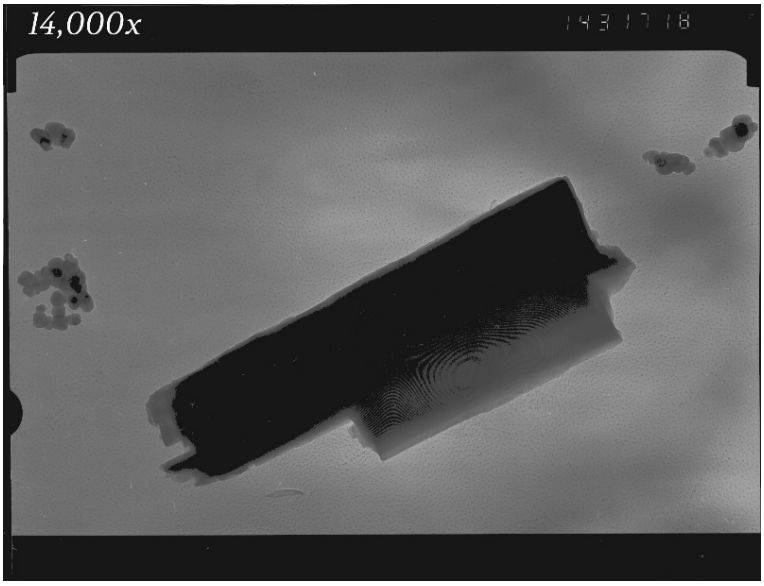
305281-4 Particle 11 20.5 x 0.18 microns Tremolite

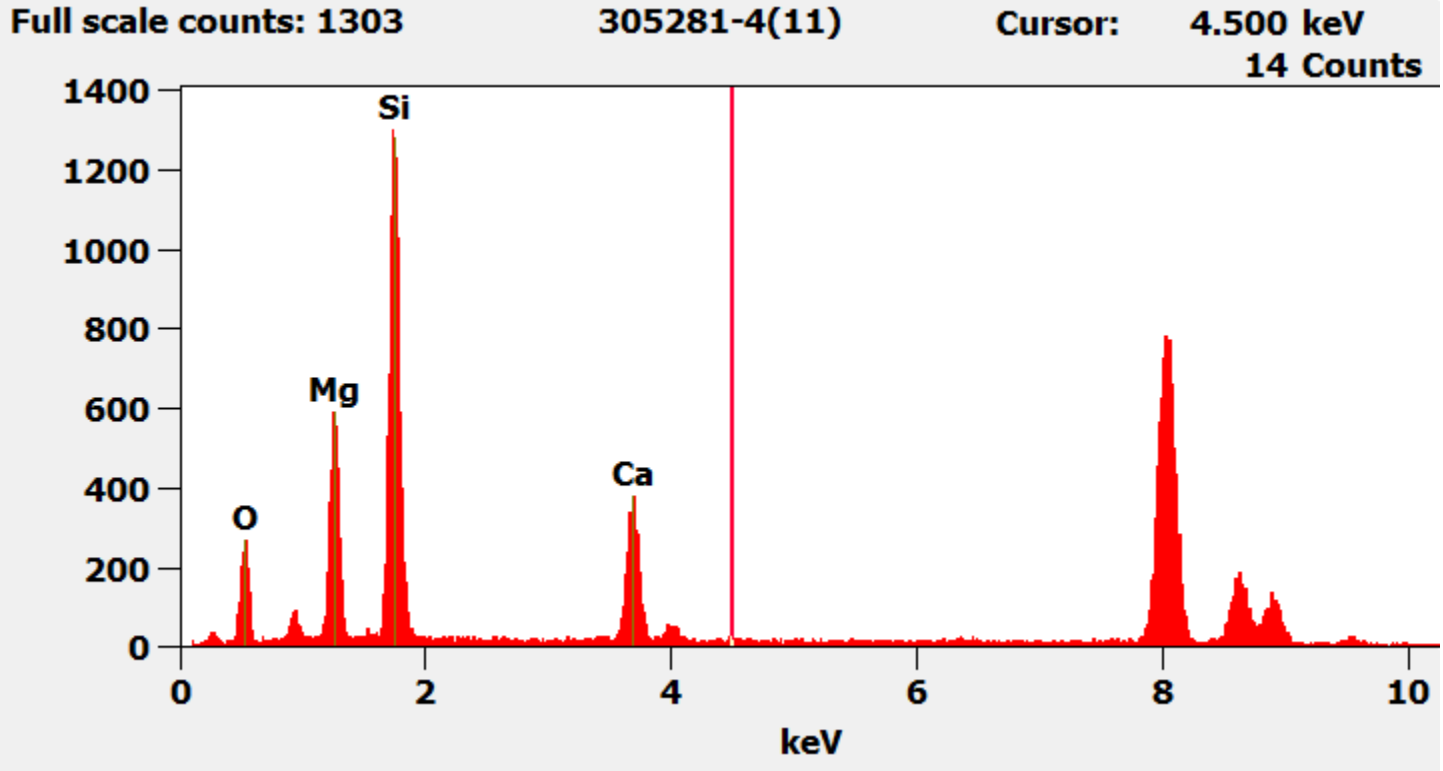




Element Line	Weight %	Weight % Error
O K	54.02	± 3.07
Mg K	14.93	± 0.54
Si K	27.96	± 0.56
Si L	---	---
Ca K	3.09	± 0.18
Ca L	---	---
Total	100.00	

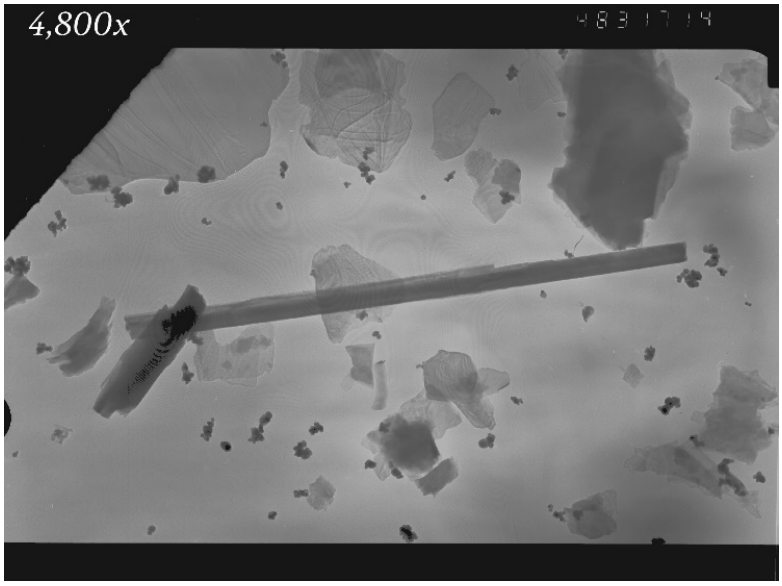
305281-4 Particle 12 4.5 x 1.7 microns Tremolite

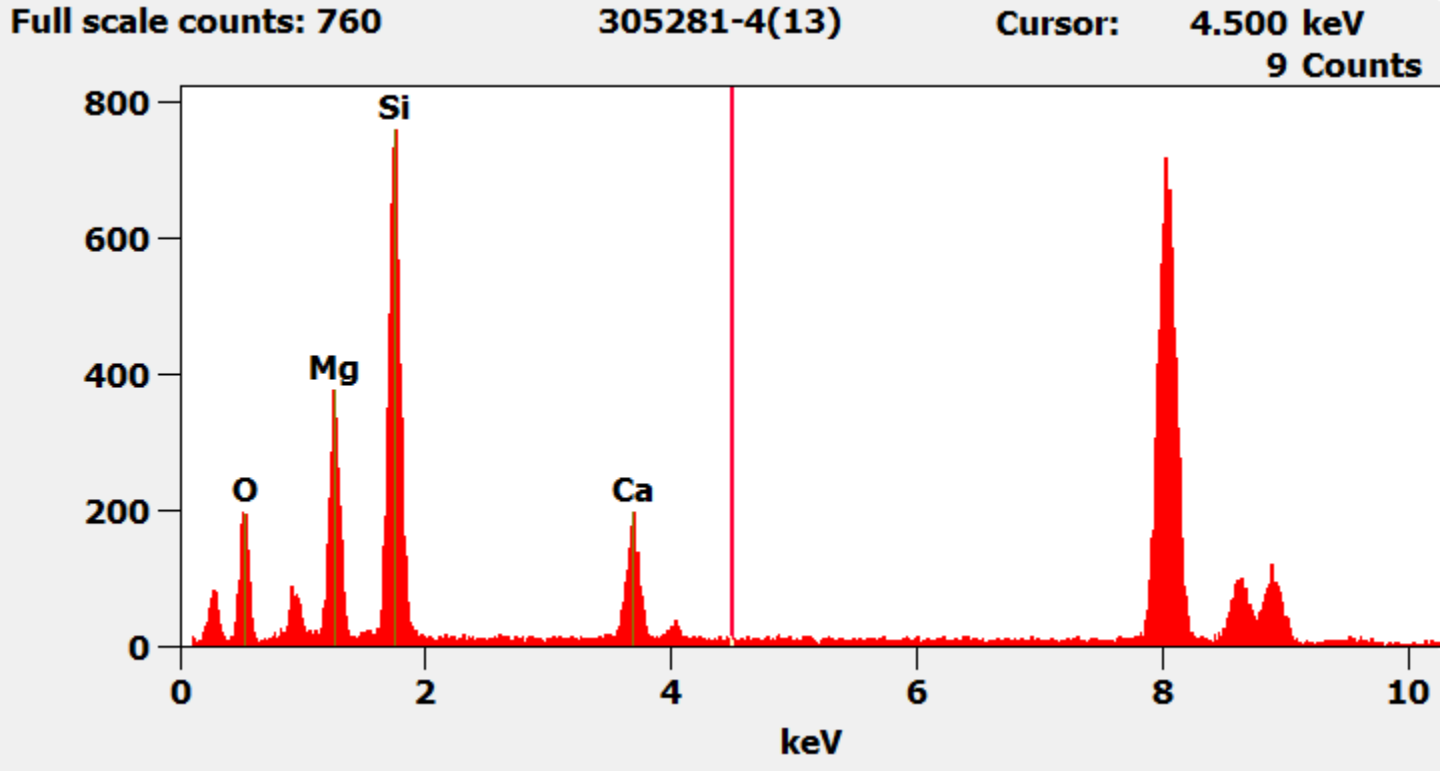




Element Line	Weight %	Weight % Error
O K	48.73	± 1.26
Mg K	17.21	± 0.28
Si K	29.82	± 0.30
Si L	---	---
Ca K	4.24	± 0.10
Ca L	---	---
Total	100.00	

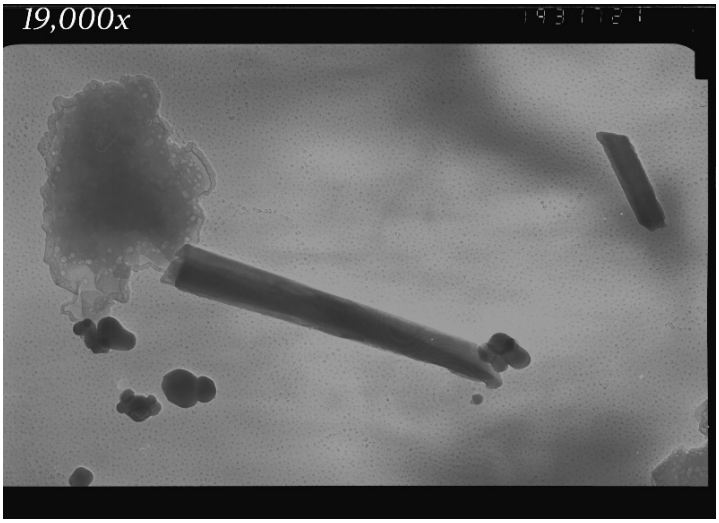
305281-4 Particle 13 & 14 4.4 x 1.3 microns & 15.6 x 0.8 microns Tremolite

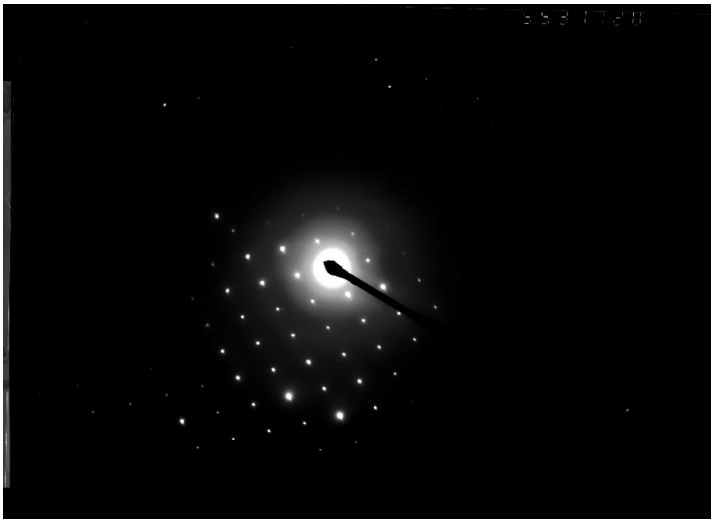


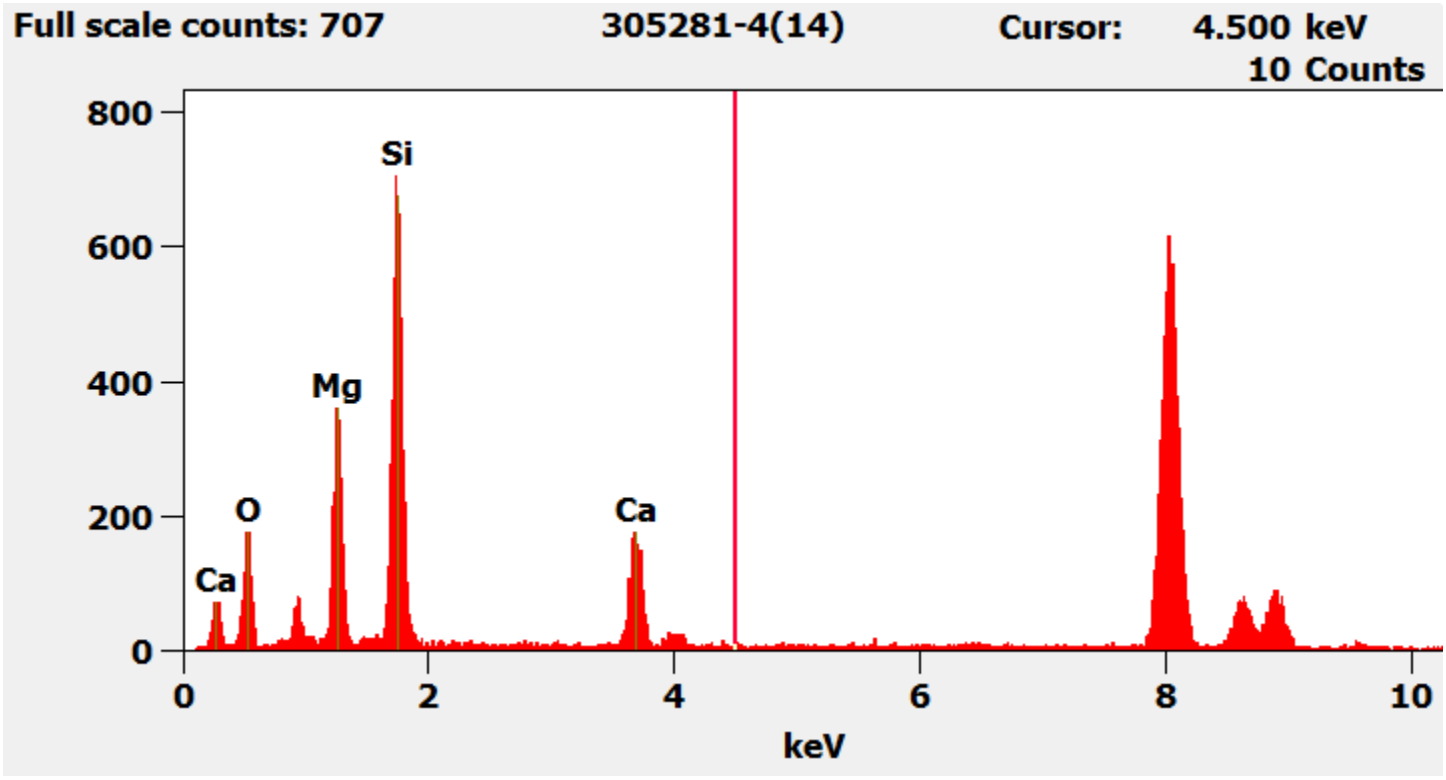


Element Line	Weight %	Weight % Error
O K	52.28	± 1.64
Mg K	17.40	± 0.35
Si K	27.41	± 0.36
Si L	---	---
Ca K	2.92	± 0.11
Ca L	---	---
Total	100.00	

305281-4 Particle 15 2.7 x 0.26 microns Tremolite

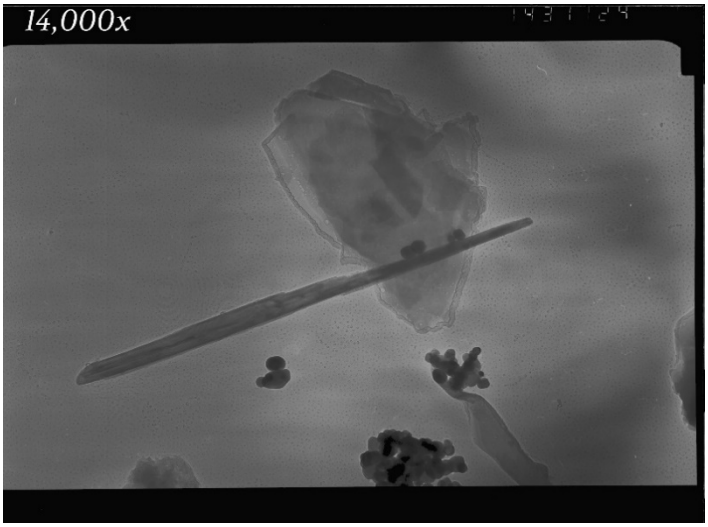


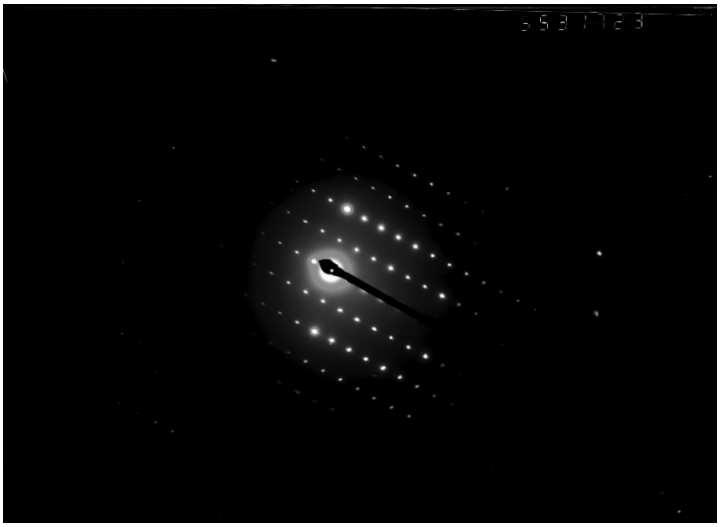


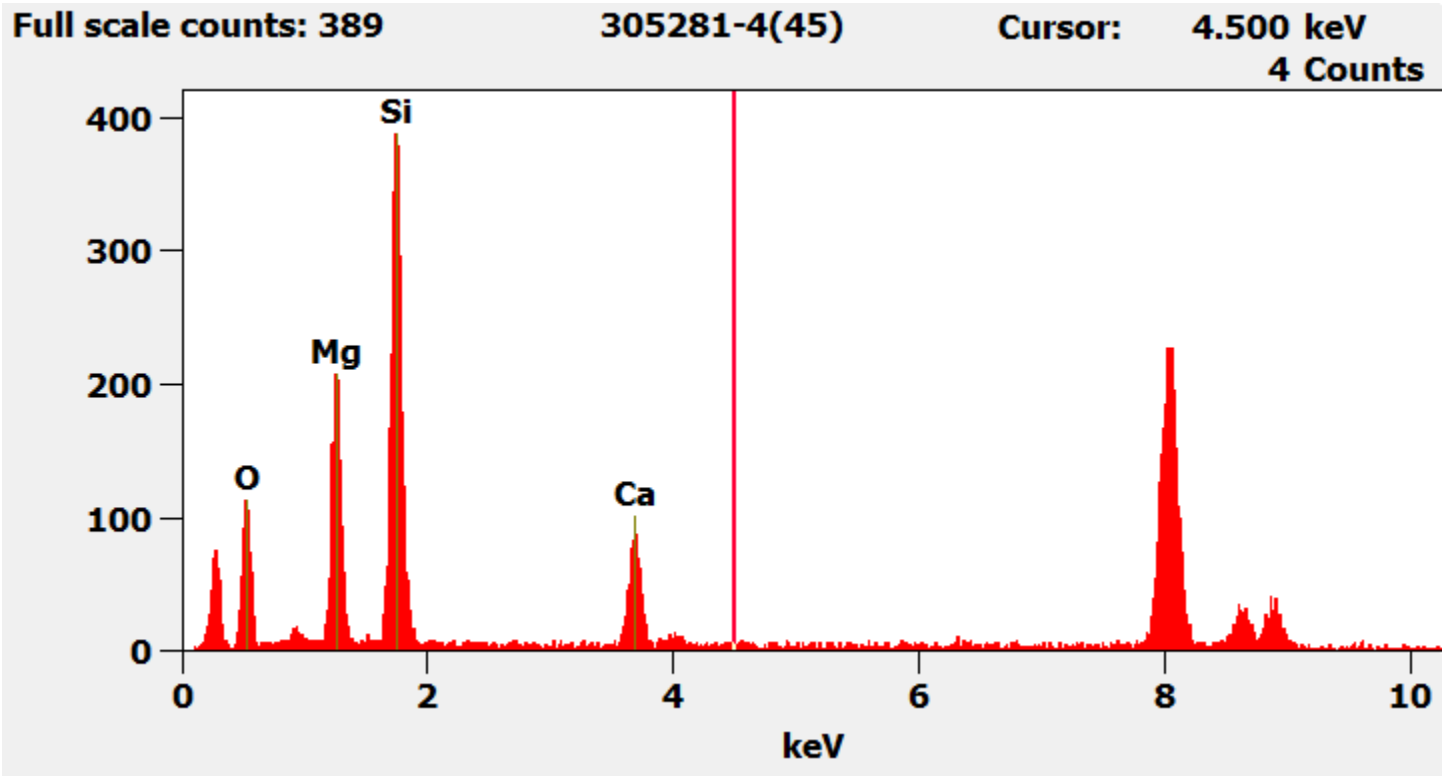


Element Line	Weight %	Weight % Error
O K	59.21	± 2.63
Mg K	17.88	± 0.68
Si K	19.08	± 0.52
Si L	---	---
Ca K	3.84	± 0.14
Ca L	---	---
Total	100.00	

305281-4 Particle 51 4.8 x 0.2 microns Tremolite







Element Line	Weight %	Weight % Error
O K	52.86	± 2.32
Mg K	17.64	± 0.46
Si K	26.86	± 0.48
Si L	---	---
Ca K	2.64	± 0.13
Ca L	---	---
Total	100.00	

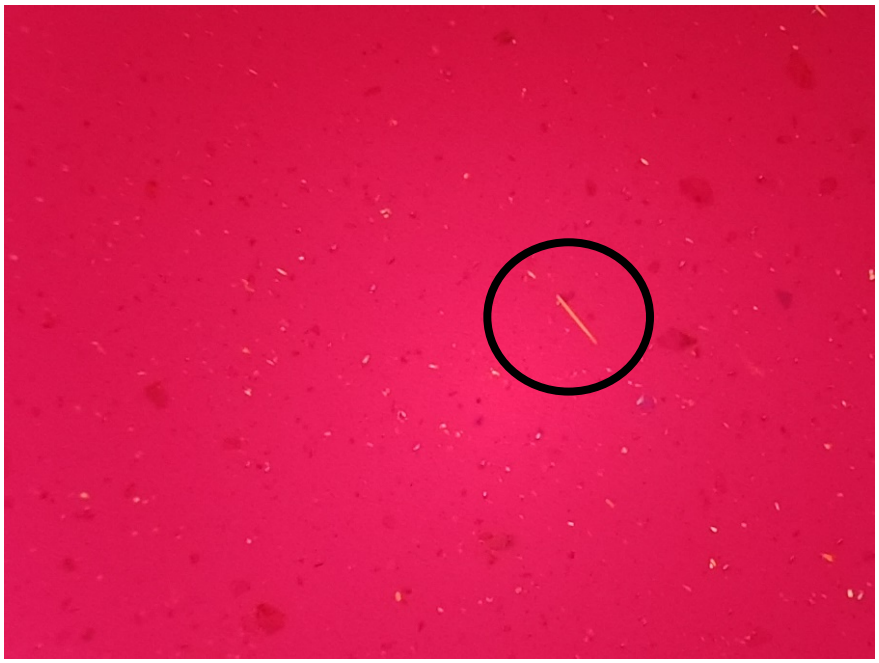
305281-5, 5A, 5B, Client Sample D31

PLM

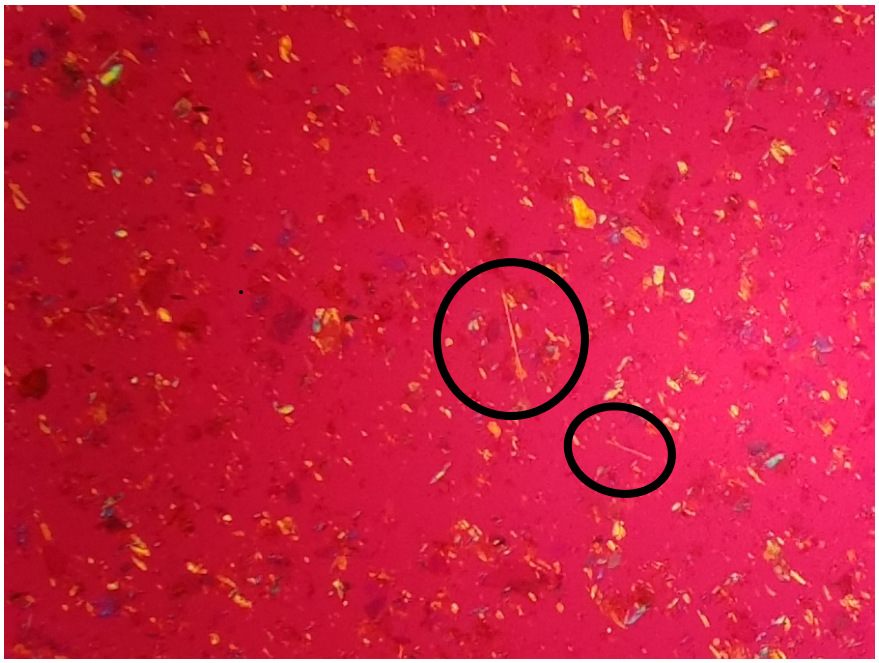
All three aliquots of sample D31 were analyzed by (b) (6) on January 31, 2019. Tremolite was observed on all three aliquots but no points were counted. No other asbestos was detected. The results were calculated using the equations detailed in the calculations section.

305281-5	<0.18% Tremolite detected
305281-5A	<0.18% Tremolite detected
305281-5B	<0.18% Tremolite detected

305281-5 Tremolite 100x



305281-5A Tremolite 100x



305281-5B Tremolite 100x

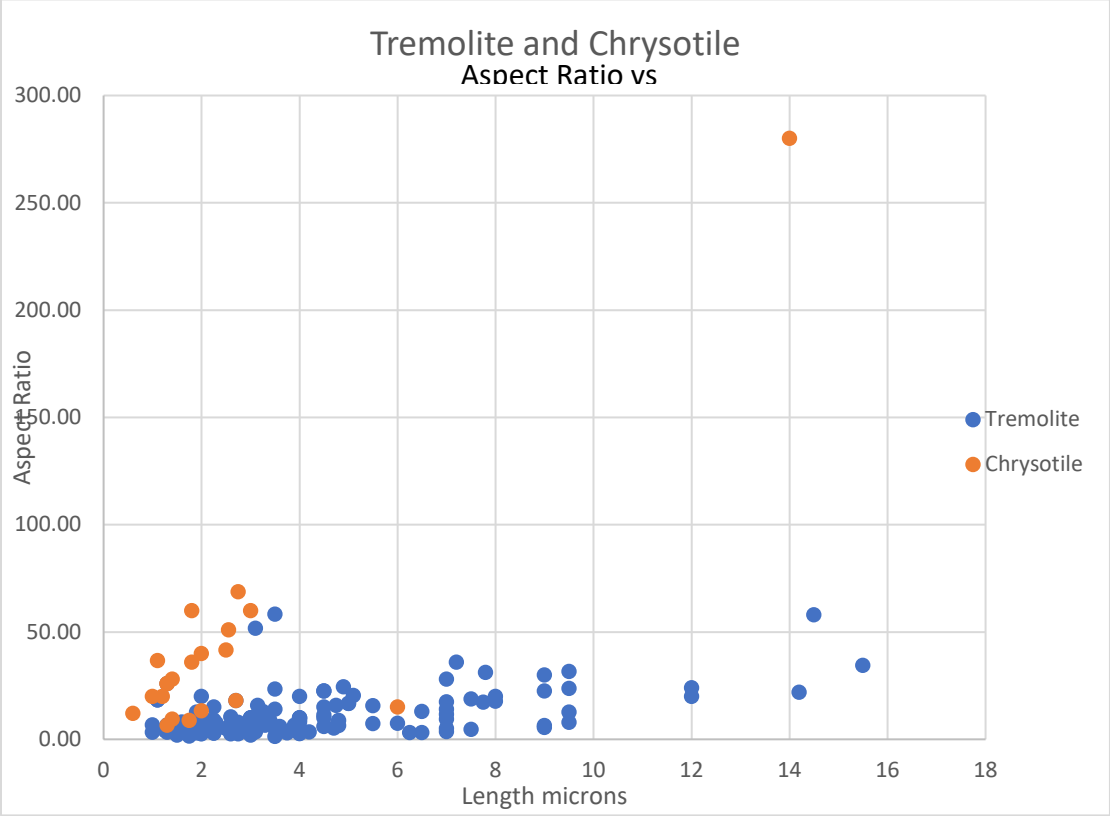
TEM

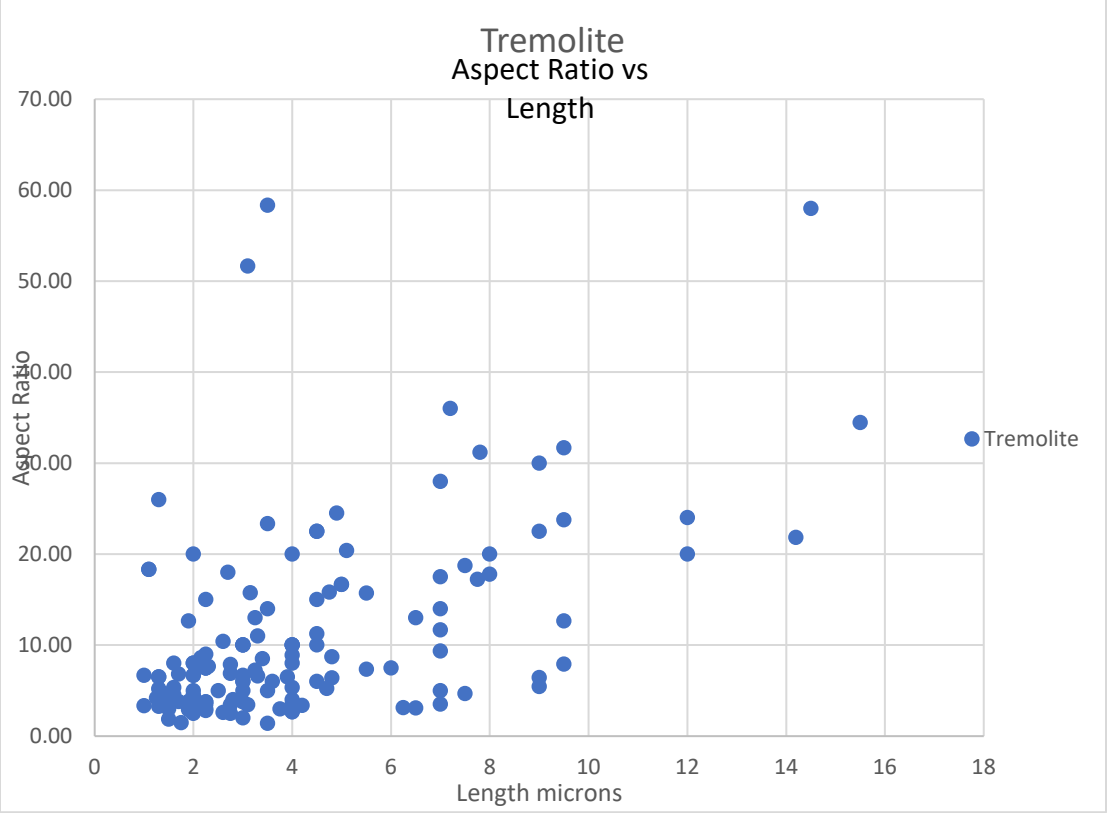
Samples 5, 5A and 5B were analyzed by (b) (6) on January 15-17, 2019. Talc is the main constituent in all three aliquots. The talc is primarily in plate form but there are also some talc fibers and ribbons. Tremolite and chrysotile was detected on all three aliquots. 20 grid openings were analyzed on all three aliquots. Sample 5 had 43 tremolite and 7 chrysotile particles counted. Sample 5A had 43 tremolite and 5 chrysotile particles. Sample 5B had 55 Tremolite and 9 chrysotile particles. The length and width of each particle was recorded. The mass of the tremolite was calculated using both the ASTM D5756 and ISO 22262-2 methods. The mass of the chrysotile was calculated using the ASTM D5756 method.

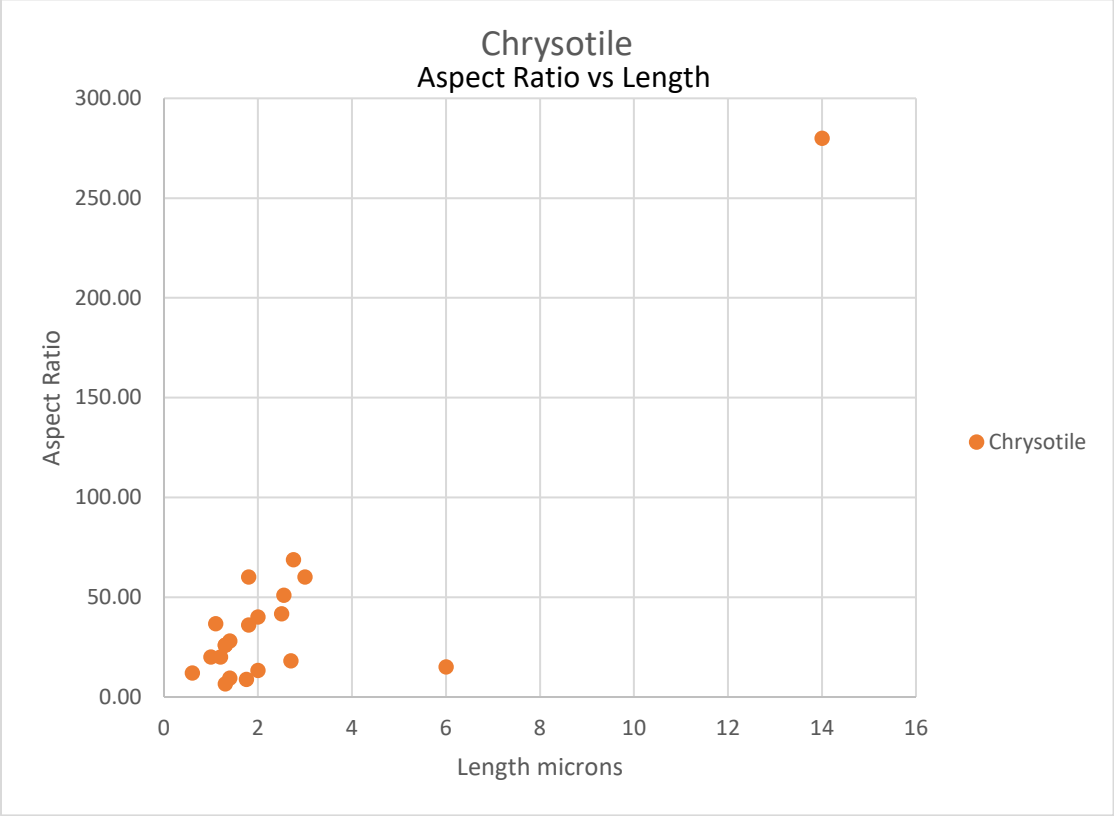
305281-5	0.156% to 0.244%
305281-5A	0.171% to 0.268%
305281-5B	0.134% to 0.210%

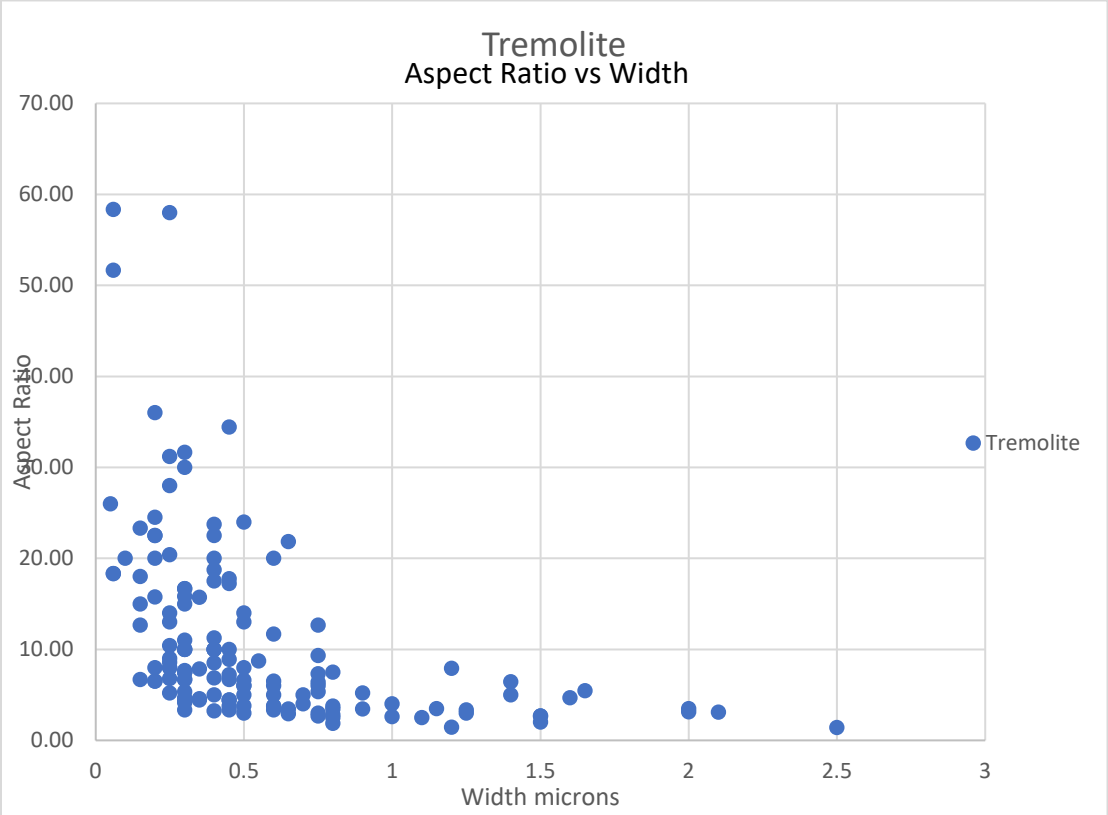
The tremolite particles varied in morphology from small blocks to very long, high length to width aspect ratio fibers. The length to width aspect ratio of particles counted ranged from 1.40 to 58.33. The mean aspect ratio is 11.0. The chemistry of the tremolite observed consisted of O, Mg, Si, and Ca. The chrysotile particles were high length to width aspect ratio fibers. The aspect ratio ranged from 6.5 to 280. The mean aspect ratio was 41.8. The chemistry of the chrysotile was O, Mg, Si, Fe.

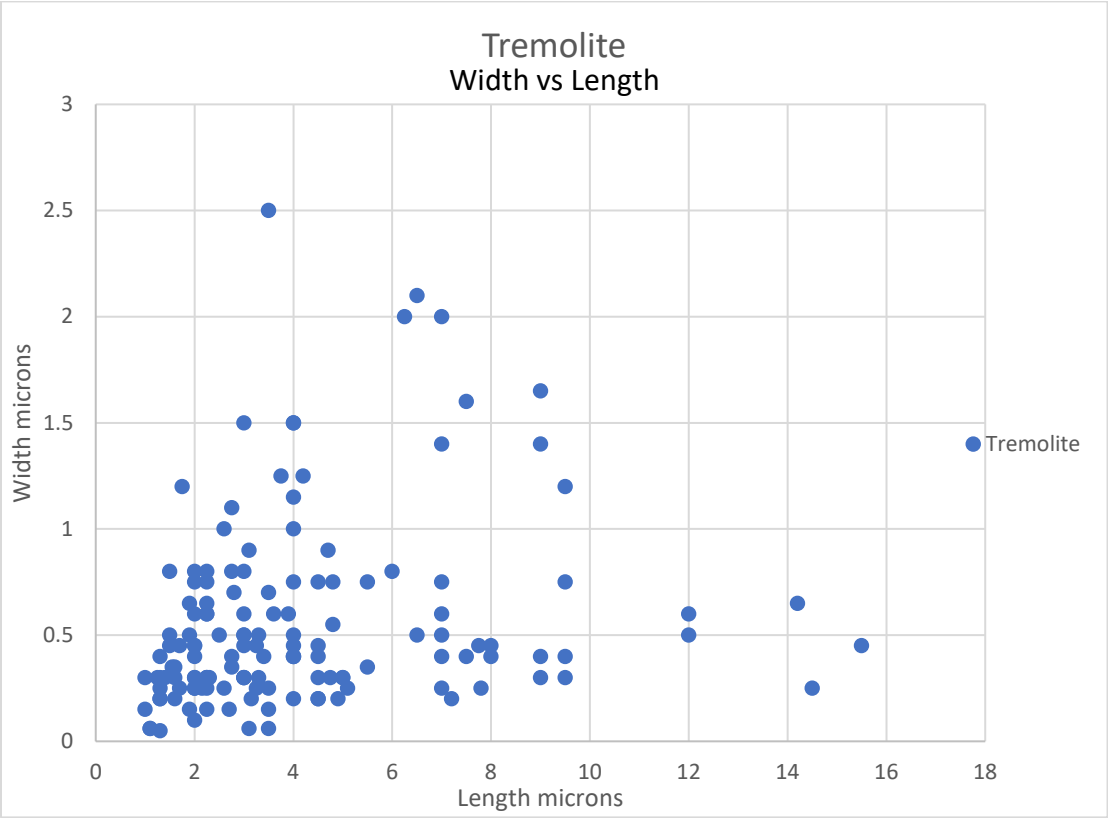
The following charts are a plot of the aspect ratio vs length for all the particles counted over all three aliquots, for the tremolite only, and for the chrysotile only.


Tremolite and Chrysotile
Aspect Ratio vs
300.00
250.00
200.00
150.00
100.00
50.00
0.00
Aspect Ratio
0
2
4
6
8
10
12
14
16
18
Length microns
Tremolite
Chrysotile


Tremolite
Aspect Ratio vs
Length
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Aspect Ratio
0
2
4
6
8
10
12
14
16
18
Length microns
Tremolite

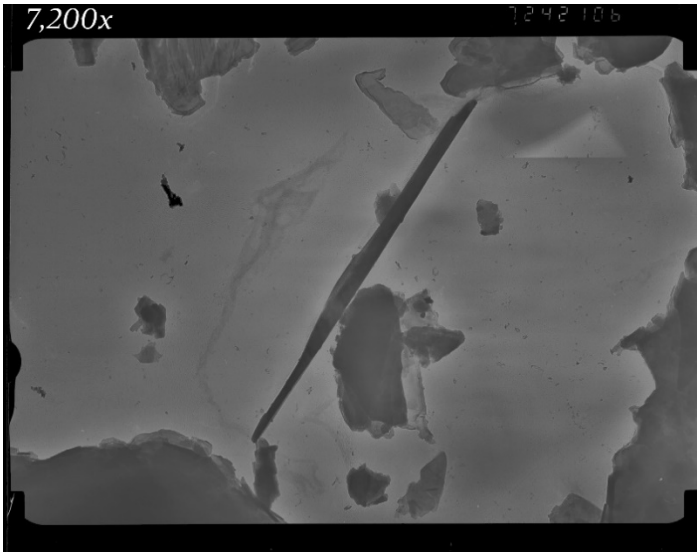

Chrysotile
Aspect Ratio vs Length
300.00
250.00
200.00
150.00
100.00
50.00
0.00
Aspect Ratio
0
2
4
6
8
10
12
14
16
Length microns
Chrysotile

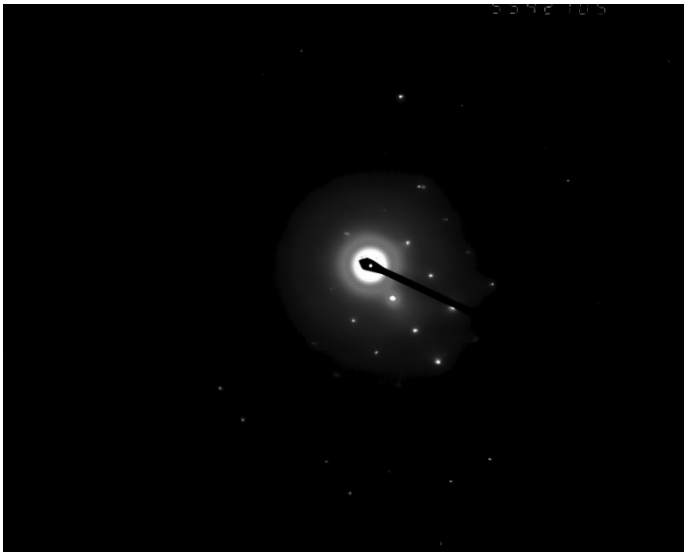

Tremolite
Aspect Ratio vs Width
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Aspect Ratio
0
0.5
1
1.5
2
2.5
3
Width microns
Tremolite

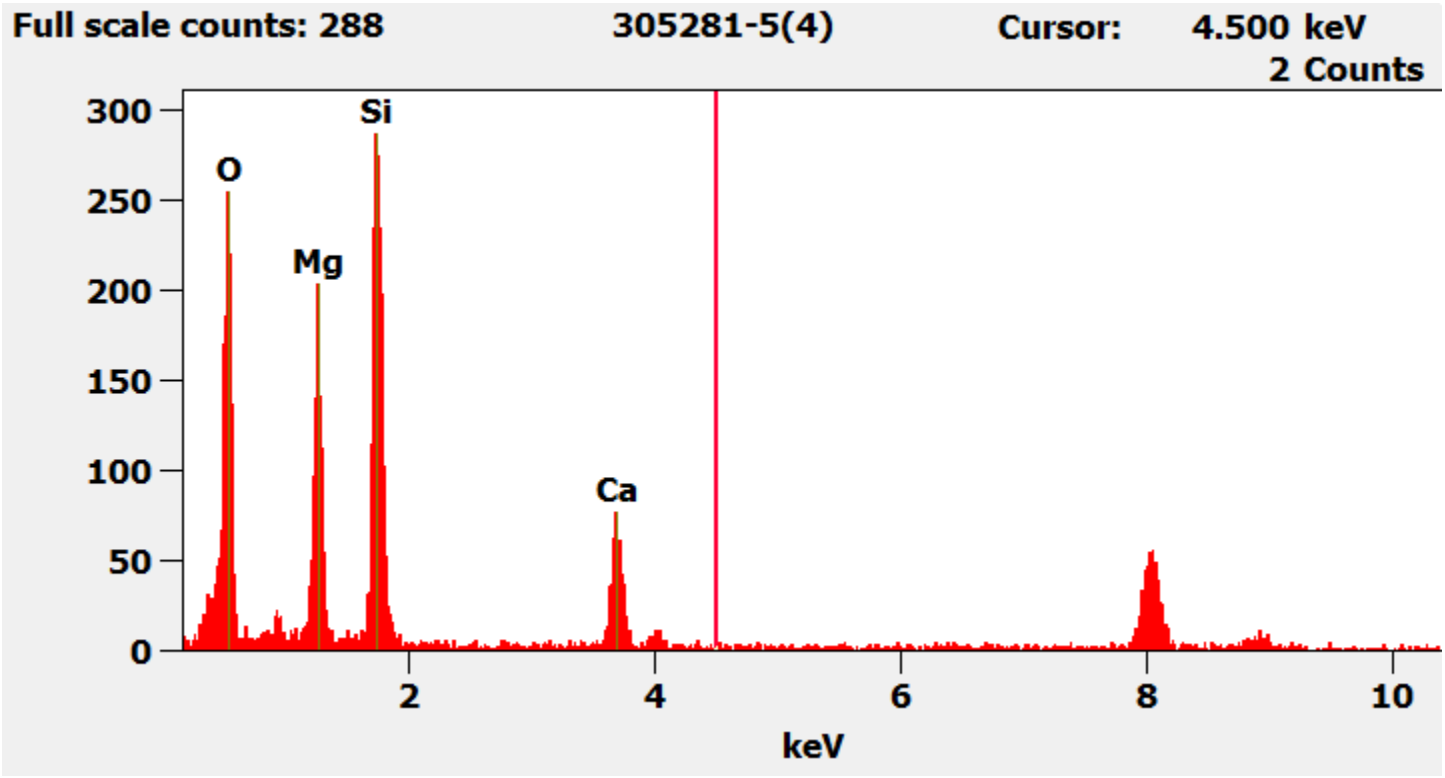


Below are pictures, diffraction patterns, and chemistry from some of the counted particles. The unidentified peaks in chemistry spectra are copper, zinc, and carbon. Those peaks are from the TEM specimen holder and specimen grid.

305281-5 Particle 3 8.0 x 0.40 microns Tremolite

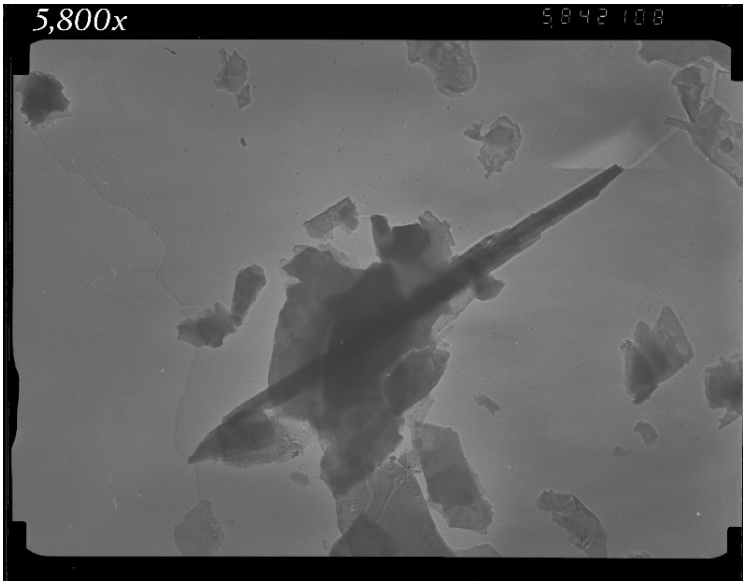


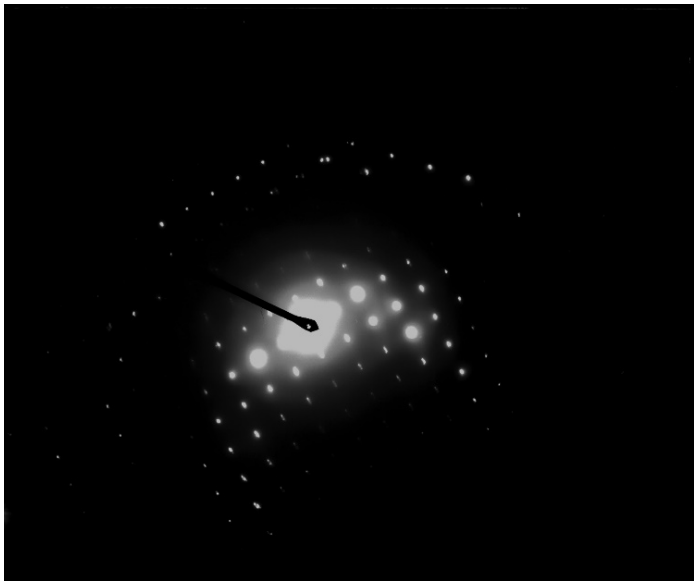


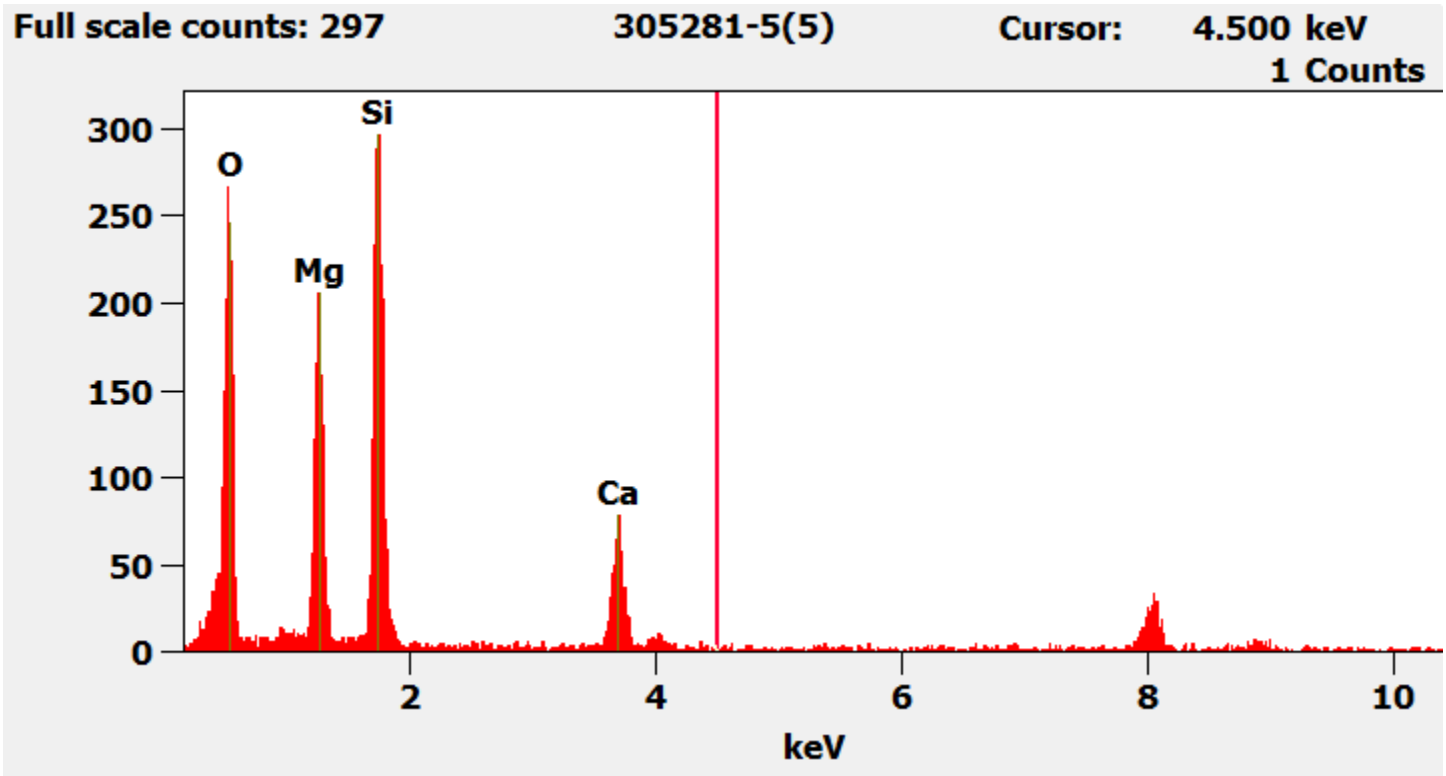


Element Line	Weight %	Weight % Error
O K	59.45	± 1.68
Mg K	16.67	± 0.51
Si K	22.15	± 0.47
Si L	---	---
Ca K	1.73	± 0.09
Ca L	---	---
Total	100.00	

305281-5 Particle 4 12.0 x 0.60 microns Tremolite

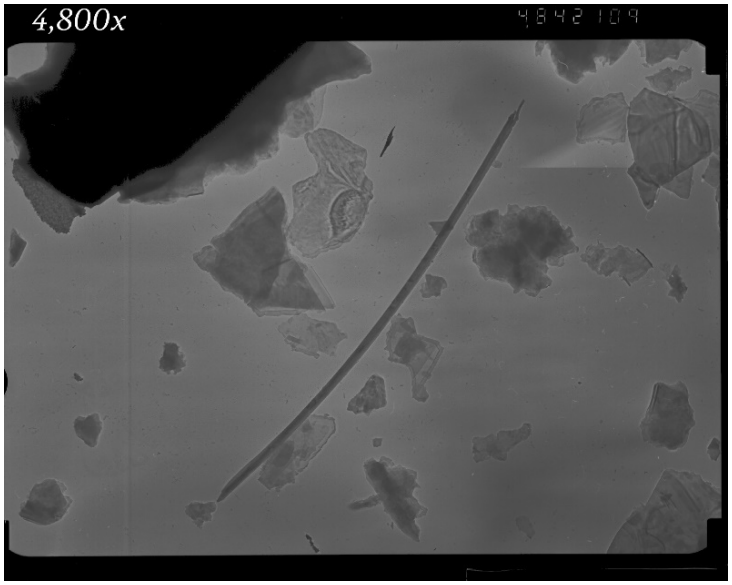


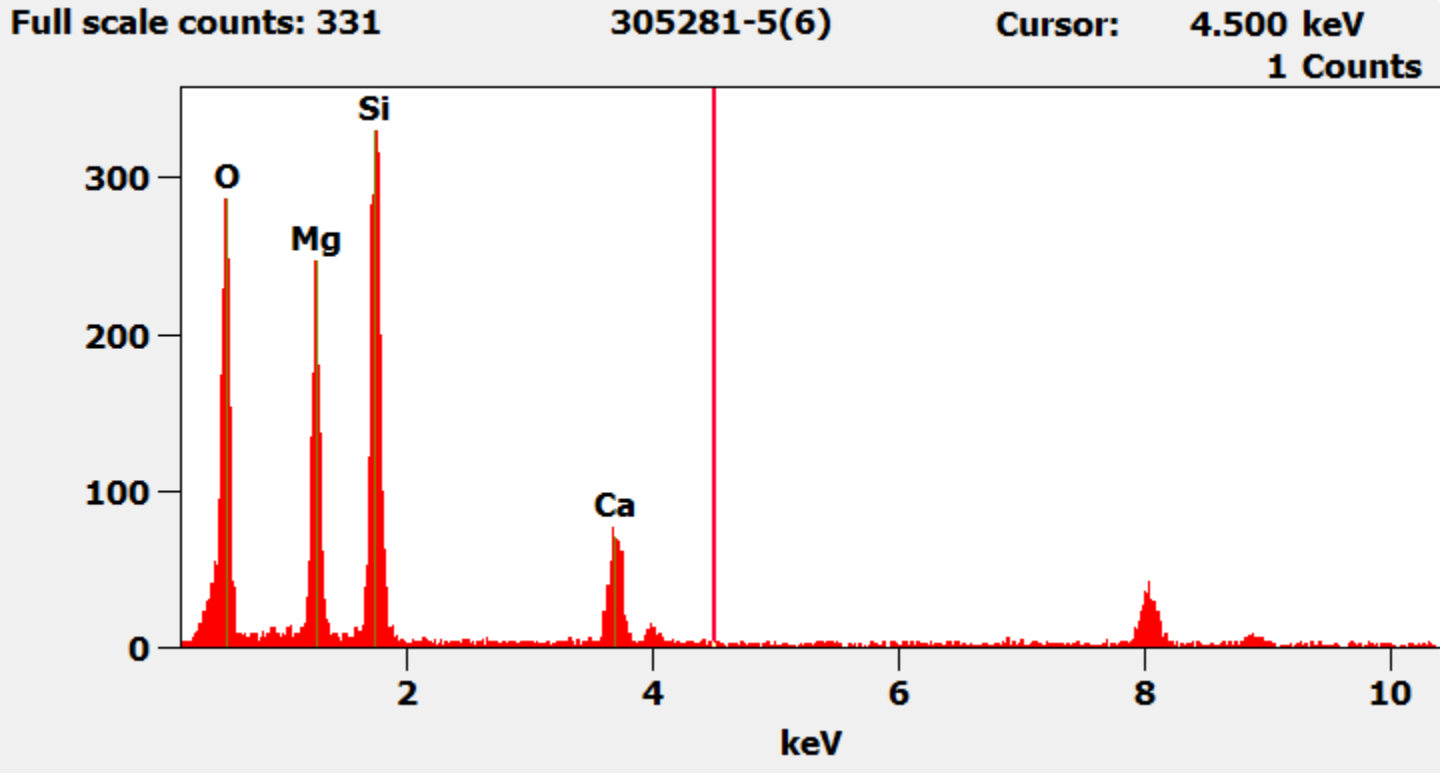




Element Line	Weight %	Weight % Error
O K	59.77	± 1.60
Mg K	17.11	± 0.51
Si K	21.58	± 0.45
Si L	---	---
Ca K	1.54	± 0.09
Ca L	---	---
Total	100.00	

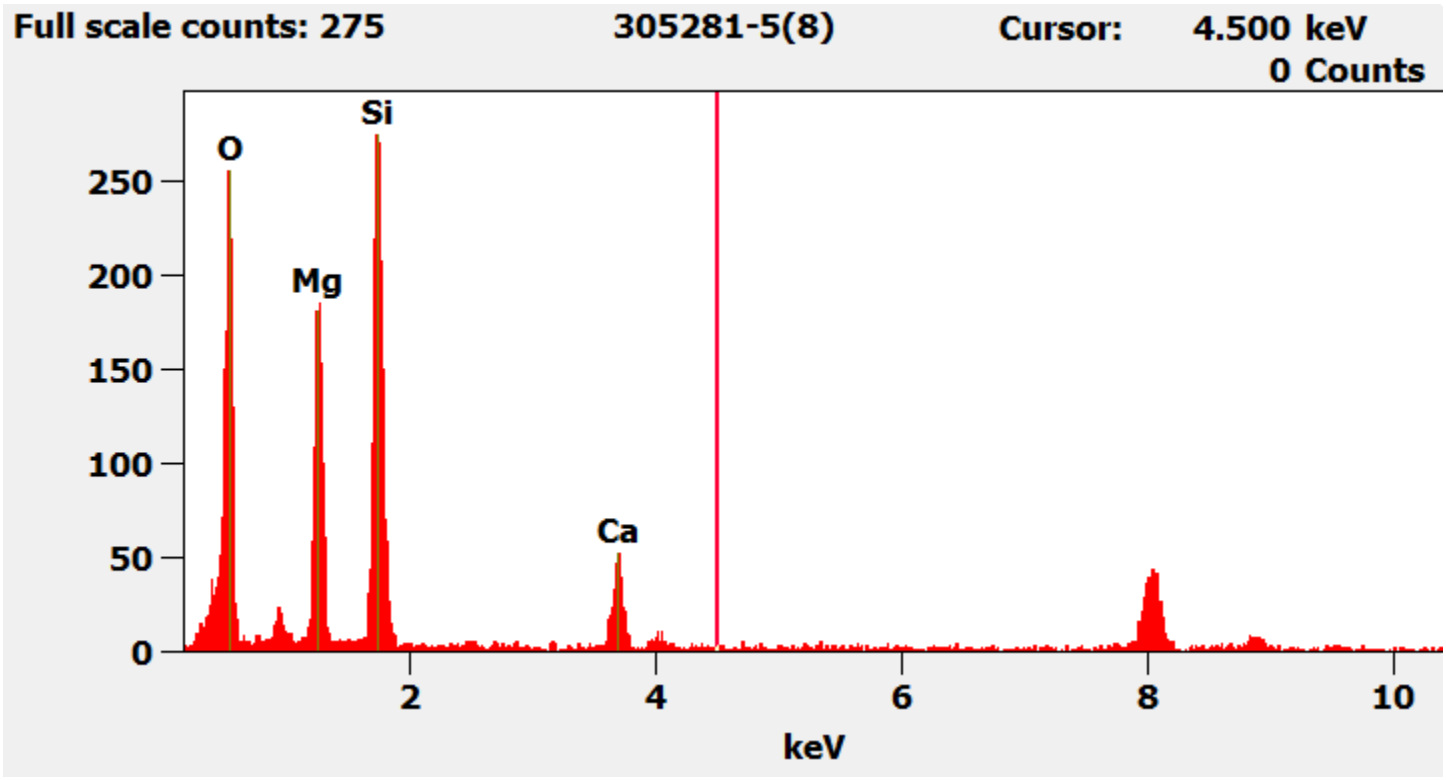
305281-5 Particle 5 12.0 x 0.60 microns Tremolite





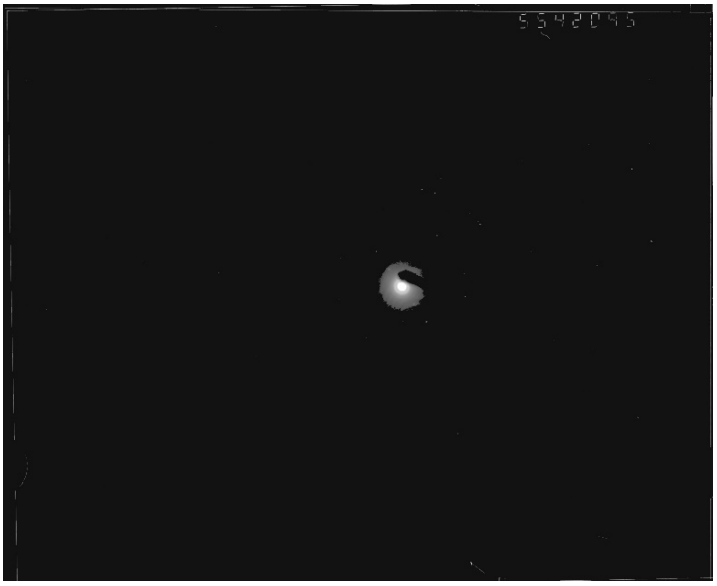
Element Line	Weight %	Weight % Error
O K	59.22	± 1.53
Mg K	17.13	± 0.47
Si K	22.06	± 0.43
Si L	---	---
Ca K	1.60	± 0.09
Ca L	---	---
Total	100.00	

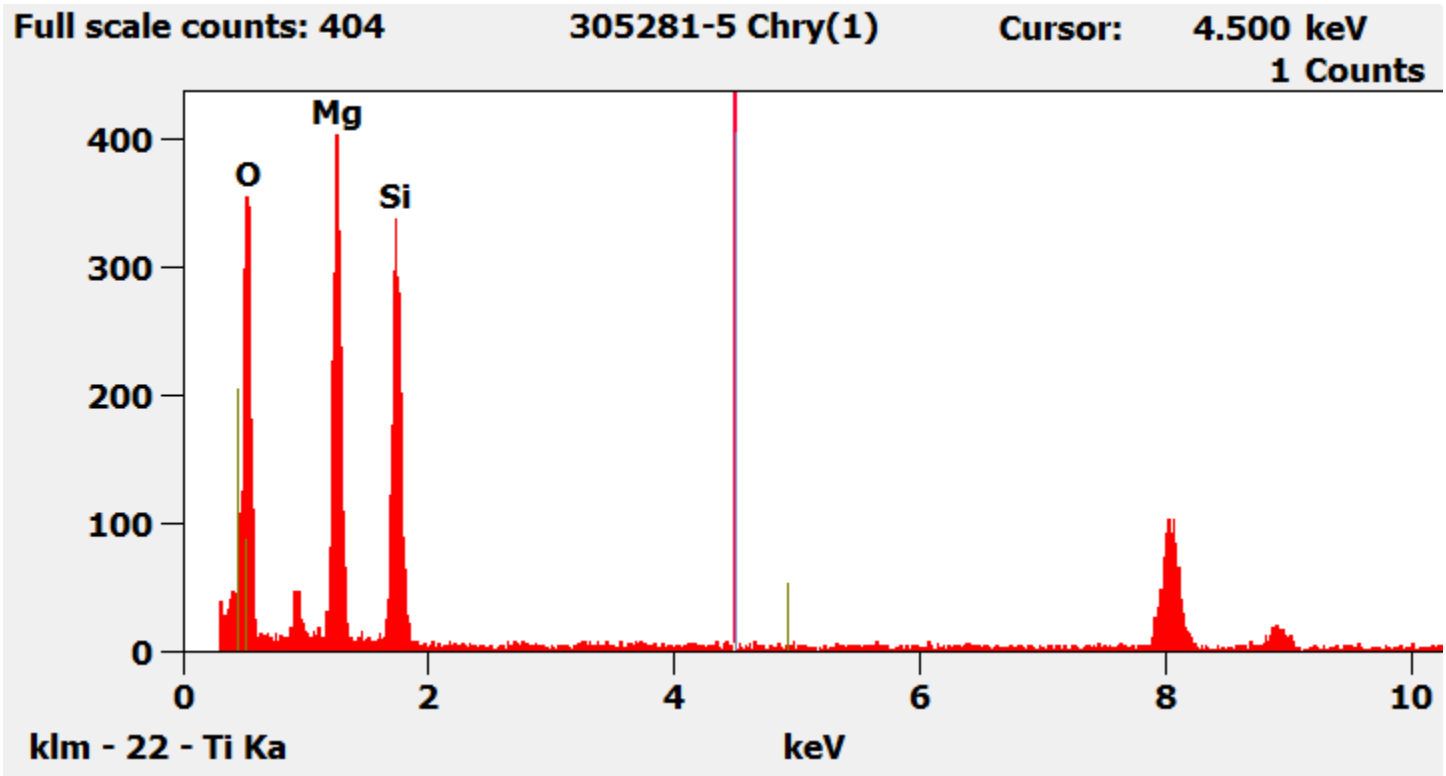
305281-5 Particle 6 1.70 x 0.25 microns Tremolite



Element Line	Weight %	Weight % Error
O K	58.34	± 2.26
Mg K	17.65	± 0.67
Si K	22.56	± 0.63
Si L	---	---
Ca K	1.45	± 0.12
Ca L	---	---
Total	100.00	

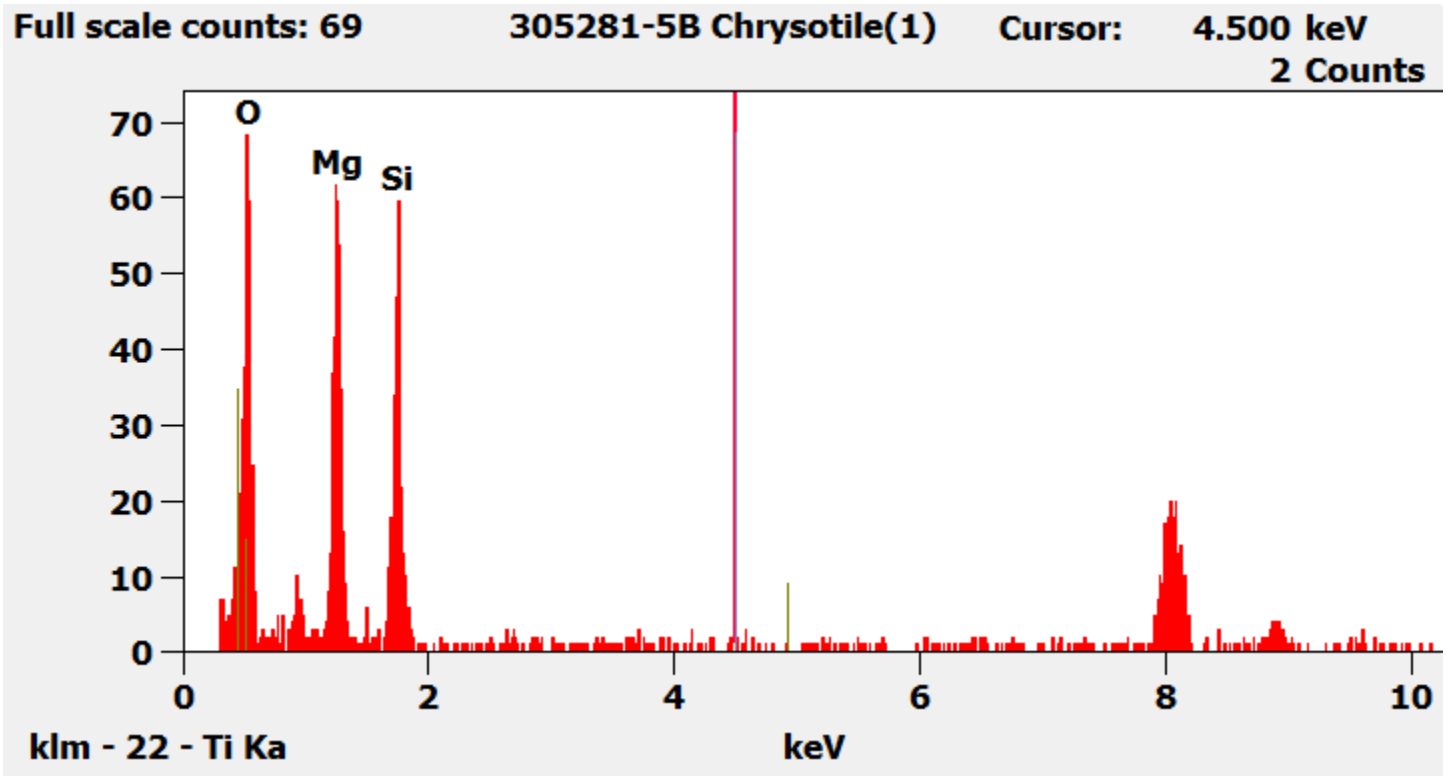
305281-5B Particle 49 1.40 x 0.15 microns Chrysotile





Element Line	Weight %	Weight % Error
O K	55.89	± 1.44
Mg K	24.04	± 0.49
Si K	20.07	± 0.42
Si L	---	---
Total	100.00	

305281-5B Particle 56 2.50 x 0.06 microns Chrysotile



Element Line	Weight %	Weight % Error
O K	60.28	± 3.44
Mg K	22.85	± 1.17
Si K	16.86	± 0.96
Si L	---	---
Total	100.00	

(b) (6)

(b) (6) (b) (6)

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(b) (6)

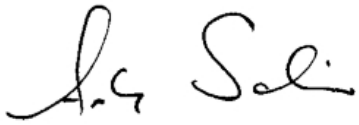
QC Discussion:
During preparation, one blank control sample and one reference control sample were prepared. These samples were prepared alongside the customer samples. The blank sample was prepared using Sigma-Aldrich Talc Powder, <10 micron and was analyzed by (b) (6) on January 31, 2019. No asbestos was detected on the blank sample. The reference sample was made from the same Sigma-Aldrich talc powder spiked with 0.4% Chrysotile. The reference sample was analyzed by (b) (6) on February 21, 2019 and found to be within acceptable limits.

Our laboratory information management systems (LIMS) randomly selected sample 305281-6/D96 for additional replicate QC analysis. Separate preparations were made for PLM and TEM analysis. The replicate QC analysis was performed by (b) (6) on January 31, 2019 for PLM analysis and by (b) (6) on February 18, 2019 for TEM analysis. The QC results matched the original analysis.

Attachments:
The following items are attached to this case narrative for your reference:

1) Sample Log-In Sheet
2) Daily PLM Scope Calibration Log
3) Refractive Index Oil Calibration Log
4) Daily TEM Scope Calibration Log
5) QC Results Summary
6) Replicate & Duplicate QC Chart for (b) (6) for samples analyzed between 9/1/2018 and 2/18/2019
7) Replicate & Duplicate QC Chart for (b) (6) for samples analyzed between 1/1/2018 and 2/18/2019
8) Replicate & Duplicate QC Chart for (b) (6) for samples analyzed between 1/1/2018 and 2/18/2019
9) Replicate & Duplicate QC Chart for (b) (6) for samples analyzed between 1/1/2018 and 2/18/2019
10) Raw Data Sheets
    a. Gravimetric Data
    b. Filtration Worksheets
    c. PLM Analysis
    d. TEM Analysis
    e. QC Samples

I certify that all information contained in this report pertaining to laboratory events, procedures, and protocols is true and accurately describes the handling of this project by AMA Analytical Services, Inc. and its personnel.



2/21/2019

Andreas Saldivar — Date
Laboratory Director