CO1-1 Status Report 2015 for B–3 Beam Port of KUR

K. Mori, H. Yoshino, Y. Iinuma, T. Fukunaga, Y. Kawabata, S. Sato[1], H. Hiraka[1], K. Iwase[2], Y. Yamaguchi[3] and T. Shiono[4]

Research Reactor Institute, Kyoto University (KURRI)
[1]High Energy Accelerator Research Organization (KEK)
[2]Department of Materials and Engineering, Ibaraki University
[3]Institute for Materials Research, Tohoku University
[4]Graduate School of Engineering, Kyoto University

INTRODUCTION: The B–3 beam port of Kyoto University Research Reactor (KUR) had long been used as a four-circle single-crystal neutron diffractometer (4CND). For the last decade, however, the 4CND was so old that its research activity on neutron science was quite low. Therefore, the 4CND needed to be replaced and a new neutron diffractometer has been required at the B–3 beam port. Also, the new neutron diffractometer (Compact multipurpose neutron diffractometer) is critical for the structural investigations of energy storage materials such as hydrogen absorbing alloys and rechargeable lithium-ion batteries. The neutron (powder) diffraction is a powerful tool to determine the positions of light elements (e.g., hydrogen and lithium) in solids. Here, we report the current status of the B-3 beam port of KUR and the preliminary neutron diffraction experiments using a hydrogen absorbing alloy.

INSTRUMENT: The compact multipurpose neutron diffractometer is now being installed on the B–3 beam port. The neutron wavelength (λ), which is monochromatized by the (220) plane of a Cu single crystal, is 1 Å. To cover the detector area ($6\ ^\circ \leq 2\theta \leq 150\ ^\circ$), 24 ^{3}He tube detectors (1/2 inch in diameter) have been prepared. The distances from the monochromator to the sample and from the sample to the detector will be 1.9 m and 1.2 m, respectively. A detector bank including the 24 ^{3}He tube detectors will be placed on an arm of the goniometer. In addition, the new beam shutter was installed at the B-3 beam port.

PRELIMINARY EXPERIMENTS: The preliminary neutron diffraction experiments were performed using the hydrogen absorbing alloy, $(Ti_{0.31}Cr_{0.33}V_{0.36})D_{1.7}$, where D is the deuterium. It is worth noting that $Ti_{0.31}Cr_{0.33}V_{0.36}$ is a null alloy for neutron scattering due to its atomic compositions (i.e., $b_c[Ti_{0.31}Cr_{0.33}V_{0.36}] = 0$; $b_c[Ti] = -3.370$ fm, $b_c[Cr] = 3.635$ fm, and $b_c[V] = -0.443$ fm), where b_c is the coherent scattering length. The crystal structure has been refined on the basis of a cubic CaF_2-type structure with $a = 4.288(1)$ Å, using X-ray diffraction with Cu $K\alpha$ radiation (see Fig. 1(b)). As shown in Fig. 1(a), we succeeded to observe several Bragg reflections for the $(Ti_{0.31}Cr_{0.33}V_{0.36})D_{1.7}$ on the B–3 beam port; which could be indexed on the basis of $\lambda = 1$ Å. Note that the Bragg reflections correspond to the D–D correlations because of the null alloy.

Furthermore, the data acquisition group of the neutron science division of KEK (KEK–KENS DAQ group) has used the B–3 beam port to assess their new ^{6}Li-glass neutron detector system, LiTA12. The LiTA12 system consists of a ^{6}Li-glass neutron detector with a multianode photo multiplier tube (MA–PMT), an amplifier, and an analog-to-digital converter (ADC) board. The B–3 beam port has a wide space around the sample position; therefore we can easily install any other system like the LiTA12 system.

IMPROVEMENT OF MONOCHROMATOR STAGE: The old Cu monochromator stage of the 4CND was removed (Fig. 2(a)), and then the new Cu monochromator stage was installed at the B–3 beam port (Fig. 2(b)). The Cu monochromator stage consists of two goniometers: rotation and swivel stages (RA07A-W and SA05B-RM, Kohzu Precision Co., Ltd.).

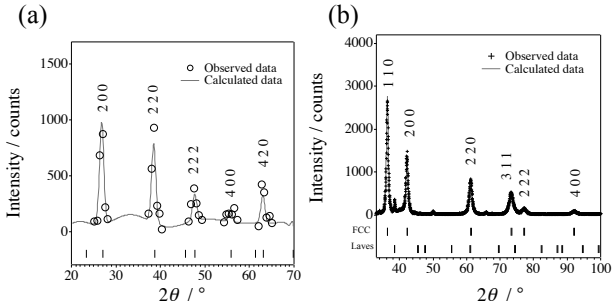

Fig. 1. Neutron and X-ray diffraction data for a hydrogen storage alloy, $(Ti_{0.31}Cr_{0.33}V_{0.36})D_{1.7}$, collected at (a) the B-3 beam port of KUR and (b) a X-ray diffractometer with Cu $K\alpha$ radiation.

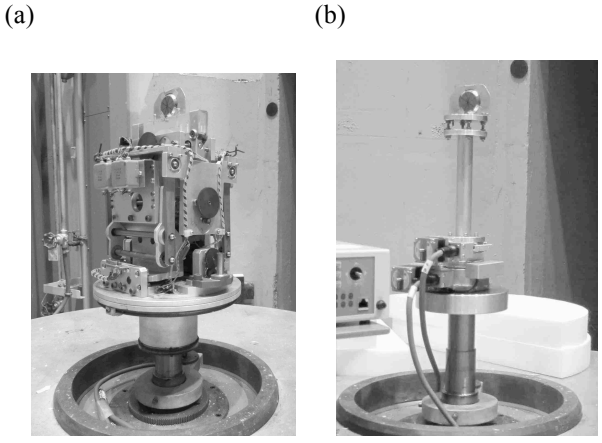

Fig. 2. Improvement of Cu monochromator stage: (a) old stage, and (b) new stage, respectively.

CO1-2 Characterization of Proteolytic Enzymes Derived from *NephilaClavata*

Y. Hidaka, K. Kouda, Y. Touyama and S. Shimamoto

Graduate School of Science and Engineering Kinki University

INTRODUCTION: Spiders trap insects using a web net. The fact that they eat them without chewing, indicate that spiders possesses highly efficient digestive enzymes [1]. Our previous studies suggest that spider's proteolytic enzymes are able to digest synthetic spider dragline amyloid fibers [2,3]. Thus, the spider protease has the potential ability to digest amyloid fibers including pathogenic β-amyloid, such as amyloid fibrils, that are associated with the development of Alzheimer's disease [1]. Therefore, we purified, identified, and characterized the enzymes derived from *Nephila Clavata*. The sequence analyses of the blotted protein on a PVDF membrane showed N-terminal amino acid residues. Based on this sequence information, we tried to clone the protease gene.

EXPERIMENTS: The spiders (*Nephila Clavata*) were collected in the field of Kyoto University Research Reactor Institute and the spider saliva including proteolytic enzymes was obtained from *Nephila Clavata* (50 heads) by electrical stimulation. The extracts were stored in a deep freezer until use.

Casein protease assay [4] of the crude spider enzymes was performed, as previously reported [1]. Peptide substrates for the assay of the protease activity of spider enzymes were chemically synthesized by the Boc solid phase method, treated with hydrogen fluoride, and purified by reversed phase HPLC [1, 4].

Based on the N-terminal sequence, evidenced by the Edoman's degradation method, we prepared generated primers for the gene cloning and RT-PCR was performed using mRNA's from gut cells or full bodies of *Nephila Clavata.*

RESULTS: Spider's digestive fluid that includes proteolytic enzymes was obtained from *Nephila Clavata* by electrical stimulation using a micropipet. The extracts were applied to SDS-PAGE and the enzymatic activity of the protein bands was estimated by a casein protease assay [4]. Two protein bands showed protease activities and their molecular weights were estimated to be approximately 21.9 and 19.5 kDa, based on the SDS-PAGE analysis, as we determined previously [1].

To determine the gene sequence of the spider protease, we tried to obtain its mRNA. For this purpose, generated primers for the gene cloning of the protease were specifically designed and RT-PCR was performed using mRNA from gut cells or full bodies of *Nephila Clavata.* Several clones were isolated and provided second PCR to produce cDNA's of spider proteases. The purified candidate cDNA's were applied to PCR again, then their cDNA sequence were directly determined. However, the sequence analysis of the cloned spider protease gene revealed that the cloned genes were non-specifically amplified during RT-PCR. Therefore, generating primers were re-designed by considering codon bias of spider. RT-PCR and following PCR produced several candidate cDNA's . Their sequence analyses is now in progress.

We also prepared the cDNA of cocoonase to estimate amyloid digestive activity and expressed it using *E. coli* cells. Recombinant cocoonase was successfully over-expressed as a inclusion body. The protein was purified and analyzed by reversed phase HPLC and MALD-TOF/MS, respectively. To activate the protein, refolding reactions were carried out under several conditions and its protease activity will be estimated using a synthetic substrates.

In conclusion, Spider protease was extracted from its digestive fluid and exhibited strong protease activity. The protease can be classified as a Ca^{2+}-dependent carboxypeptidase, based on the results of protease inhibition assays. Spider genes were amplified using generating primers, base on the N-terminal amino acid sequence, by RT-PCR and determined by the direct sequencing method. However, DNA sequence analysis indicated that the cloned DNA was non-specifically amplified during RT-PCR. The cloning of spider protease genes is in progress. In addition, we also prepared the cDNA of the protease, cocoonase andover-expressed it using E. coli expression system. Recombinant cocoonase was successfully refolded as a soluble protein. Its biological activity will be estimated and provide a critical insight to understand the amyloid digestive activity of spider and Bombyx mori.

REFERENCES:

[1] M. Fujiwara *et al.*, *Peptide Science*, **2013,** 447-448 (2014).

[2] S. Zheng, *et al.*, *Appl. Spectrosc.,* **43**, 1269-1272 (1989).

[3] Y. Hidaka, *et al.*, *Biopolymers*, **96**, 222-227 (2011).

[4] C. Dicko, *et al.*, *Biochemistry*, **43**, 1269-1272 (2004).

CO1-3 Development of a Plano-elliptical Supermirror with Metallic Substrates

M. Hino, T.Hosobata[1], N.L.Yamada[2], Y.Yamagata[1], S. Takeda[3], J. Guo[1], S.Morita[4], T.Oda, M.Furusaka[2] and Y. Kawabata

Research Reactor Institute, Kyoto Univ., Japan
[1] RAP, RIKEN, Japan
[2] IMSS, KEK, Japan
[3] Grad. Sch. of Eng., Hokkaido Univ., Japan
[4] Tokyo Denki Univ. Japan

INTRODUCTION: Progress of neutron optical devices is very significant, however, it is still very difficult for neutron aspherical focusing mirror. The metallic substrate is robust and ductile, able to withstand high radiation irradiation and high-temperature. It is therefore possible to install them even at a place close to the neutron target and moderator. Furthermore, it is possible to fabricate a large focusing supermirror by combining multiple seg-mented mirrors with mechanical fastening entailing the usage of screw holes and fixture tabs. The big problem is required surface roughness for neutron mirror. It is smaller than 0.5 nm even for *m*=3 supermirror coating and the mirror size is large. Here *m* is the maximum crit-ical angle of the mirror in units of critical angle of natural nickel. In order to realize large ellipsoid neutron focusing supermirror, we are doing a lot of trials. In this study, we show the neutron reflectivity of a large plano-elliptical supermirror with metallic substrates.

EXPERIMENTS: We fabricated a large plano-elliptical metallic substrates with electroless nickel-phosphorus (NiP) plating, based on the technology using ultrahigh precision cutting with correction processing, followed by mechanical precision polishing. We manufactured a two segment large plano-elliptical supermirror with *m*=3 supermirror coating for the Soft Interface Analyzer (SOFIA) at the Materials and Life Science Experimental Facility (MLF) of Japan Particle Accelerator Research Complex J-PARC [1]. Because the area limitations of machines used for manufacturing and supermirror coat-ing. To realize sample focusing optics, the focusing mir-ror was installed at an equidistant position between first slit and the sample position. One focal point was at the first slit. The other was at the sample position. The major axis of the mirror was designed to be 2150 mm. The mi-nor axis was designed to reflect neutrons to use the typi-cal wavelength band (> 0.2 nm) for reflectivity measure-ments at SOFIA. For the mirror coating, the *m*=3 super-mirror was chosen to keep as high a reflection angle as possible while simultaneously keeping the neutron reflec-tivity sufficiently high. Therefore, the minor axis was chosen as 21.5 mm to reflect the neutrons on the mirror with an incident angle of 10 mrad. The mirror length was chosen as 550 mm to avoid interference with other de-vices installed to SOFIA. The width was 60 mm, as de-termined from the maximum beam size width of 50 mm. The manufacturing, polishing and cleaning of the metallic substrate were conducted at RIKEN [2]. The supermirror coating was conducted with KUR-IBS [3]. The neutron experiments were conducted at the SOFIA reflectometer at J-PARC/MLF.

RESULTS: Figure1 shows neutron reflectivity of the 10 measured locations in the plano-elliptical supermirror with metallic substrates. Typical surface roughness of the top surfaces of metallic substrates was approximately 0.3 nm rms using a white light interferometer (NewView 7200; Zygo Corp.).Using the KUR-IBS, m=3 NiC/Ti supermirrors were coated onto the substrates surface. The maximum neutron reflectivity of the supermirror on the elliptical metallic substrates was 0.8, which is almost identical to that with flat glass substrates. However, the average of neutron reflectivity was not so high because of surface contamination resulting from inadequate cleaning.

The figure error shape was 4.6 μm P–V. The slope error was 33 μrad rms. The focusing supermirror was installed at the SOFIA neutron reflectometer, showing high neutron reflectivity and giving minimal beam width of 0.34 mm in FWHM. Because of the large beam divergence accepted by the mirror, the count rate with the focusing mirror was 3.3 times higher than that obtained using conventional two-slit collimation[2].

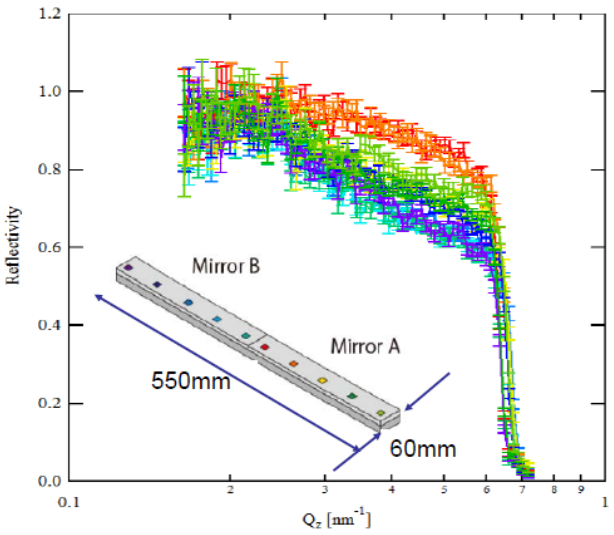

Fig. 1. Neutron reflectivity of the 10 measured locations in the plano-elliptical supermirror with metallic substrates. The focusing mirror consists of two segements (Mirror A and B).

REFERENCES:

[1] N.L.Yamada, *et al.*Euro.Phys.J.Plus 126(2011), 108.
[2] S.Takeda, *et al.*, Optics Express (2016), in press..
[3] M.Hino, *et al.*, Nucl. Instr. and Meth., **797**(2015) 265.

CO1-4 Neutron Irradiation Effect of High-density MoO_3 Pellets for Mo-99 Production (3)

T. Ishida, Y. Suzuki, K. Nishikata, M. Yonekawa, Y. Kato, A. Shibata, A. Kimura, Y. Matsui, K. Tsuchiya, T. Sano[1], Y. Fujihara[1] and J. Zhang[1]

Neutron Irradiation and Testing Reactor Center, JAEA
[1]Research Reactor Institute, Kyoto University

INTRODUCTION: JAEA has a plan to produce ^{99}Mo by (n, γ) method ((n, γ)^{99}Mo production), a parent nuclide of ^{99m}Tc. In 2014, irradiation test was carried out with the high-density molybdenum trioxide (MoO_3) pellets in the hydraulic conveyer (HYD) of the Kyoto University Research Reactor (KUR) and the ^{99m}Tc solution was extracted from the solution of irradiated MoO_3) pellets. In this study, the qualification of ^{99m}Tc solution was evaluated.

EXPERIMENTS: The high-density MoO_3 pellets were irradiated in the HYD of the KUR. After the irradiation tests, the irradiated MoO_3 pellets were transported from KUR to JMTR-HL. The irradiated pellets were dissolved with 6M-NaOH solution (Mo Solution) in the Lead Cell and the ^{99m}Tc was extracted from the Mo solution by the solvent extraction method using methyl ethyl ketone (MEK). Two types of the alumina columns were prepared for the extraction of ^{99m}Tc solution. At first, MEK containing ^{99m}Tc was passed through a basic alumina column for removing impurities such as Mo and H_2O. Next, the MEK was flown through the acidic alumina column in order to lead the whole ^{99m}Tc. Finally, saline (0.9%-NaCl) was passed the acidic alumina column and ^{99m}Tc solution was collected as a product. The inspection of ^{99m}Tc solution was carried out using pH meter, ICP-AES, HPLC, germanium detector, and so on.

RESULTS: After the dissolution of irradiated MoO_3 pellets and extraction of ^{99m}Tc solution, ^{99}Mo and ^{99m}Tc activities were measured in the Mo solution and ^{99m}Tc solution by the germanium detector. Table 1 shows the result of ^{99m}Tc recovery rates from the Mo solution [1]. The recovery rate of the 1st and 2nd runs achieved the target values (>80%).

Table 1 ^{99m}Tc recovery rates from the Mo solution

No.	Activity of ^{99m}Tc (Bq)	Recovery rate (%)
1st run	1.09×10^9	82.3
2nd run	8.53×10^8	95.7

Table 2 shows the qualification of ^{99m}Tc solutions. In this table, the standard values were decided under the framework of Strategic Program for Basic Nuclear Research launched by the MEXT. Radionuclidic purities and radiochemical impurities were measured and the values obtained satisfactory results. Especially, ^{92m}Nb observed in the Mo solution as impurities, but ^{92m}Nb was not detected in the ^{99m}Tc solutions of 1st run and 2nd run. Figure 1 shows the γ-ray spectrum of Mo solution and ^{99m}Tc solution. MEK content was lower than that of the standard values (5,000ppm). Osmotic pressure of the ^{99m}Tc solutions was almost the same values of that of the saline and endotoxin was not detected. From the results, the high purity ^{99m}Tc solutions were obtained by this method.

Table 2 Qualification of ^{99m}Tc solutions

Sample / Items	Standard Values	1st run	2nd run
pH	4.5 – 7.0	5.5	5 - 6
Radionuclidic purities	≦0.015%	^{99m}Tc(only)	^{99m}Tc(only)
Radiochemical impurities	≦5(%)	2.0	-
MEK content (ppm)	≦5000	404	-
Al content (ppm)	≦10	-	<5
Osmotic pressure (mosm)	270 - 300	286	297
Endotoxin	N.D.	N.D.	-

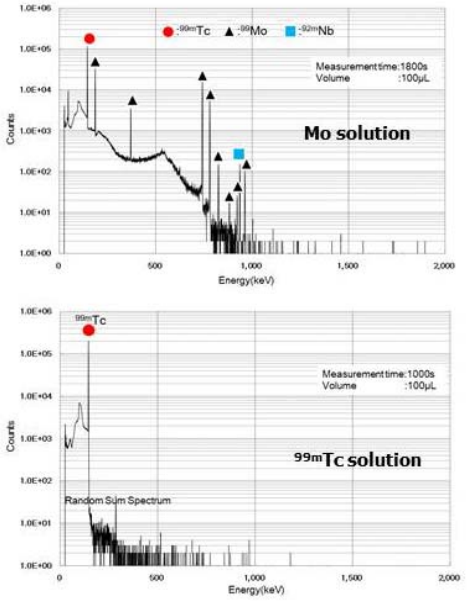

Figure 1 γ-ray spectrum of Mo and ^{99m}Tc solutions.

CONCLUSION: After the irradiation test of the high-density MoO_3 pellets in the KUR, ^{99m}Tc was extracted from the Mo solution. The qualification of ^{99m}Tc solution by the solvent extraction method was satisfied the standard values for ^{99m}Tc radiopharmaceutical products.

REFERENCES:

[1] K. Nishikata, et al., Neutron Irradiation Effect of High-density MoO_3 pellets for Mo-99 Production (2), KURR Progress Report 2014, p109.

CO1-5 Development of High Spatial Resolution Cold / Ultra-cold Neutron Detector

N. Naganawa, S. Awano[1], M. Hino[2], K. Hirota[1], H. Kawahara[1], M. Kitaguchi[1], K. Mishima[3], and A. Umemoto[1]

Institute of Materials and Systems for Sustainability, Nagoya University
[1]Graduate School of Science, Nagoya University
[2]Research Reactor Institute, Kyoto University
[3]KEK

INTRODUCTION: We have been developing cold/ultra-cold neutron detectors with spatial resolution of several ten nanometers, by using fine-grained nuclear emulsion [1, 2] with its AgBrI crystal size of 40nm, and nuclides which emit heavily ionizing particles after absorbing neutrons with high absorption cross section. One of the aim is to measure the position distribution of ultra-cold neutrons in the quantized states in the earth's gravitational field for the test of gravitational inverse-square law and equality of gravitational and inertial mass, as done in previous studies [3, 4], but more precisely, by 1 or 2 orders of magnitude.

The extremely high resolution of several ten nanometers should be realized with a 10nm-thick ^{10}B layer, coated with nuclear emulsion. When a neutron is absorbed in the ^{10}B layer, an alpha particle and a ^{7}Li will be emitted. After the development, emulsion layer will be observed with an optical microscope. A track of an alpha particle or a ^{7}Li starting from the absorber layer will be seen in the emulsion layer just next to the absorber layer. To decide the absorption point, the track will be extrapolated into the absorber layer. Then, ambiguity of the absorption position will be limited by the thickness of the absorber.

In this experiment, we made the detector and exposed to cold neutrons to test the detection principle.

EXPERIMENTS: A detector was made in the following procedure. A layer of ^{10}B with thickness of 10nm was sputtered on a base, a piece of silicon wafer, at KURRI by M. Hino. The ^{10}B layer was coated with fine-grained nuclear emulsion melted at the temperature of 40 degrees centigrade. Thickness of the emulsion layer coated was 10micron. After drying, it was packed in a light-tight bag made of thin layers of aluminum and polyethylene.

The detector was exposed to cold neutrons with energy of 10meV in average at BL05 in J-PARC MLF. After the development, observation was done by an optical microscope with an epi-illumination system.

RESULTS: As shown in Fig. 1, tracks from absorption events were clearly seen at the depth around the surface of the ^{10}B layer.

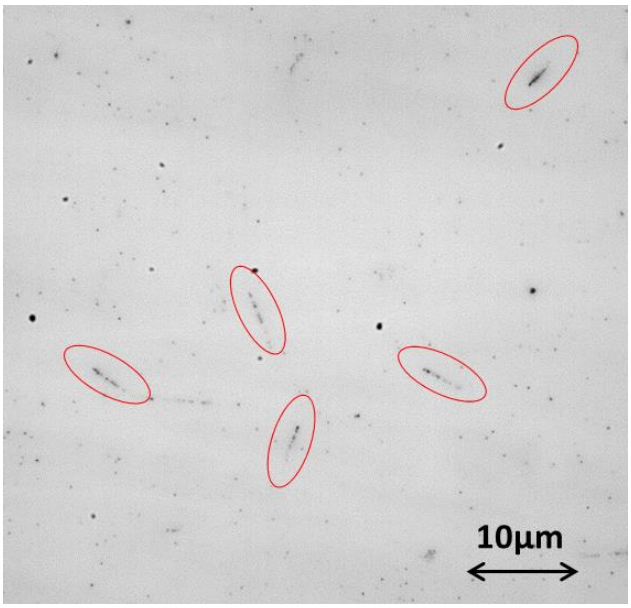

Fig.1. Tracks from absorption of neutrons by ^{10}B are surrounded by red ellipses.

But, we found tracks from absorptions by ^{10}B also inside the emulsion layer apart from the ^{10}B layer. This showed that some of ^{10}B moved into emulsion layer. ^{10}B layer turned out to be instable when it is coated directly by nuclear emulsion.

We are now developing methods to stabilize it.

REFERENCES:

[1] T. Naka and M. Natsume, Nuclear Instruments and Methods A 581 (2007) 761?764
[2] T. Naka, T. Asada, et al., Nuclear Instruments and Methods A 718 (2013) 519?521
[3] V. V. Nesvizhevsky et al., NATURE |VOL 415 | 17 JANUARY 2002 |.
[4] G. Ichikawa et al., PhysRevLett.112.071101 (2014)