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(12) **United States Plant Patent**
Nakagoshi et al.

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(54) **BUNASHIMEJI MUSHROOM NAMED
'HKHM25'**

(50) Latin Name: *Hypsizygus marmoreus*
Varietal Denomination: **HKHM25**

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(JP)

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 183 days.

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(22) Filed: **Mar. 8, 2022**

(65) **Prior Publication Data**

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US 2023/0148465 P4 May 11, 2023

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A01H 15/00 (2006.01)

(52) **U.S. Cl.**
USPC **Plt./394**
CPC **A01H 15/00** (2013.01)

(58) **Field of Classification Search**
USPC **Plt./394**
See application file for complete search history.

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(74) *Attorney, Agent, or Firm* — Taro Yaguchi

(57) **ABSTRACT**

The present variety of Bunashimeji mushroom named
'HKHM25' was cultivated by the gathering and repeated
breeding of Bunashimeji mushrooms having thick and elas-
tic stems, dark cap color, strong cap roll, and a large
mushroom size, has a high quality and enhanced cultivation
stability. This edible mushroom is exquisite in stability,
reproducibility and uniformity when being produced.

25 Drawing Sheets

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BACKGROUND OF THE INVENTION

This invention relates to a new and distinct variety of
Bunashimeji mushroom, *Hypsizygus marmoreus* (Peck)
H.E.Bigelow. This new variety named 'HKHM25', culti-
vated by repeated breeding of Bunashimeji mushroom hav-
ing thick and elastic stems, dark cap color, strong cap roll,
and a large mushroom size, has a high quality and enhanced
cultivation stability, and ensures presentable stability, repro-
ducibility, and uniformity.

The annual production of Bunashimeji mushrooms is
approximately 120,000 tons in 2020, making it the second
most consumed edible mushroom after enokitake mushroom
in Japan. However, since Bunashimeji mushrooms have
become available in large quantities in the market,
Bunashimeji mushrooms with improved flavor, quality and
shelf life has been pursued, which led to the development of
'Hokuto 18gokin' by our company. Further, our company
has been conducting a wide variety of studies for the more
stable cultivation and better quality of Bunashimeji mush-
rooms. Our company has developed a 'marmo22go' mush-
room which is thicker, whiter, and has larger mushroom than
'Hokuto 18gokin'.

Further, using 'marmo22go' as a parent, as a result of
continuing breed improvement by cross breeding to improve
stability and quality, 'HKHM25', which has thicker stems
and stronger cap roll, as compared to 'marmo22go', was
developed, confirming its stability, reproducibility, and uni-
formity.

SUMMARY OF THE INVENTION

The present invention is a new and distinct variety of
mushroom plant characterized particularly by having thick
and elastic stems, dark cap color, strong cap roll, and a large

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mushroom size, which can be cultivated by gathering and
repeated breeding of fungal strains having a stability, repro-
ducibility and uniformity when being produced. This novel
and distinct variety of mushroom is identified as
'HKHM25'.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a phylogenetic tree illustrating the anteced-
ents of 'HKHM25'.

FIGS. 2A and 2B respectively show front and back
images of a dual-culture of 'HKHM25'.

FIGS. 3A and 3B respectively show front and back
images of a dual-culture of 'HKHM25' and 'Hokuto
18gokin'.

FIGS. 4A and 4B respectively show front and back
images of a dual-culture of 'HKHM25' and 'marmo22go'.

FIGS. 5A and 5B respectively show front and back
images of a fungal flora of 'HKHM25' grown in a petri dish.

FIGS. 6A and 6B respectively show front and back image
of a fungal flora of 'Hokuto 18gokin' grown in a petri dish.

FIGS. 7A and 7B respectively show front and back
images of fungal flora of 'marmo22go' grown in a petri dish.

FIG. 8 shows an image of cultivation area of 'HKHM25'.

FIG. 9 shows an image of cultivation area of 'Hokuto
18gokin'.

FIG. 10 shows an image of cultivation area of
'marmo22go'.

FIG. 11 shows an image of a fruit body of 'HKHM25'.

FIG. 12 shows an image of a fruit body of 'Hokuto
18gokin'.

FIG. 13: shows an image of a fruit body of 'marmo22go'.

FIG. 14 shows an image of density and surface color of
fungal flora in cultured hyphae of 'HKHM25'.

FIG. 15 shows an image of density and surface color of fungal flora in cultured hyphae of 'Hokuto 18gokin'.

FIG. 16 shows an image of density and surface color of fungal flora in cultured hyphae of 'marmo22go'.

FIG. 17 shows an image of development of aerial hypha of 'HKHM25'.

FIG. 18 shows an image of development of aerial hypha of 'Hokuto 18gokin'.

FIG. 19 shows an image of development of aerial hypha of 'marmo22go'.

FIG. 20 shows an image of size and distribution of mottle of 'HKHM25'.

FIG. 21 shows an image of size and distribution of mottle of 'Hokuto 18gokin'.

FIG. 22 shows an image of size and distribution of mottle of 'marmo22go'.

FIG. 23 shows an image of shape and thickness of stipe of 'HKHM25'.

FIG. 24 shows an image of shape and thickness of stipe of 'Hokuto 18gokin'.

FIG. 25 shows an image of shape and thickness of stipe of 'marmo22go'.

DETAILED DESCRIPTION OF THE INVENTION

'HKHM25' has been asexually reproduced by tissue culture, at HOKUTO CORPORATION in Japan. The history of the 'HKHM25' mushroom in terms of improvement period and the like are set forth in the following chronological list of each stage of variety improvement:

December 2014: Cultivation of 'MH025615' strain.

January 2017: Cultivation of 'M11025617' ('marmo22go') strain.

December 2019: 'MH025615' and 'MH025617' ('marmo22go') strains were crossed and a strain with thick stem, strong cap roll, and good quality among the obtained strains was selected as 'MH025633'. We then repeated the cultivation test to distinguish the strains.

March 2021: After repeated cultivation tests, since distinguishability, stability, and uniformity were confirmed, the strain was named 'HKHM25' and its cultivation was completed. Applied for registration of a new variety to the Ministry of Agriculture, Forestry and Fisheries of Japan.

The above crossing is summarized in the phylogenetic tree illustrated in FIG. 1.

The 'HKHM25' mushroom has the following characteristics: thick and elastic stems, dark cap color, strong cap roll, and overall large size.

(1) Comparison with Existing Variety by Dual Culture

Dual culture was performed for the 'HKHM25' mushroom and a similar variety to examine whether or not a zone line is formed.

Study Method:

As an examination method, a potato dextrose agar medium was used, and the 'HKHM25' mushroom and the similar variety were inoculated thereon face to face at an interval of 3 cm, and then culture was performed at 25° C. for 28 days to examine whether or not a zone line was formed.

Strains used for the comparison between present variety, 'HKHM25' and other varieties:

'HKHM25': Present variety

'Hokuto 18gokin': Variety similar to the present variety

'marmo22go': Parent variety of the present variety

Results:

Zone lines were formed between 'HKHM25' and all other co-cultured varieties

(TABLE 1, FIGS. 2 to 4)

This clearly shows that the present mushroom is a new variety.		
HKHM25	Similar varieties	
	Hokuto 18gokin	marmo22go
	+	+

+ is present and - is absent.

*Zone line formation was not observed in the dual culture between 'HKHM25' strains.

(2) Growth Characteristics of 'HKHM25'

Study Method:

After inoculating an agar piece of the 'HKHM25' having a diameter of 5 mm and an agar piece of the similar variety having a diameter of 5 mm on a potato dextrose agar medium, preculture was performed at 25° C. for 4 days so as to make the regeneration of hyphae equal (about 10 mm in diameter), and then culture was performed for 7 days at intervals of 5° C. between 5° C. and 30° C. An average daily hyphae growth rate was calculated based on a hyphae growth rate for 7 days of the culture. Also, the optimum culture period for 'HKHM25' is 80 days, and the number of growing days at that time is 23.1 days.

Results:

'HKHM25' had the fastest average daily hyphae growth rate at 20° C. In addition, HKHM25 had slower mycelial growth rate than 'Hokuto 18gokin' and 'marmo22go' at each temperature zone (Table 2).

(3) Morphological Characteristics of the 'HKHM25' Mushroom in a Cultivation Example

Cultivation Method:

Container: An 850 polypropylene bottle (Capacity: 850 ml, diameter 58 mm) was used.

Culture medium: Conifer sawdust, corn cob, rice bran and wheat bran were mixed at the dry weight ratio of 7:3:8:2, and the water content was adjusted to 65%. The culture medium was filled up to the brim of the bottle at the rate of 540±20 g per bottle, and was sterilized at high pressure.

Starter culture: About 20 ml of sawdust starter cultures per bottle was inoculated.

Culture: Culture was performed at 22° C. for 50 to 90 days at 70% moisture.

Growth: After completing the culture, the inoculum is removed, and shifted to a growing room. Development was conducted under a temperature of 15±1° C., at humidity of 95% or about 2,000 ppm CO₂ density. Also, light was not particularly irradiated until the first 14th day, then irradiated with about 500 to 1,000 Lx. The mushroom is harvested when the cap in the center of the stump has sufficiently opened.

Cultivation Results:

Table 2 shows the characteristics of the 'HKHM25' and specific difference in characteristics as compared with the similar variety when culture was performed under the abovementioned conditions.

In addition, according to R.H.S. Colour Chart, the color of the central part of cap: 199A, the color of the peripheral area of cap: 199C, the color of gill: 158C, the color of stripe: 155B. All color references are from The R.H.S. Colour Chart

of The Royal Horticultural Society of London (R.H.S.), fifth edition published in 2007. Also, the descriptions are from mushrooms that are on the 24th day.

TABLE 2

Fungus characteristics Table of <i>Hypsizygus marmoreus</i> (Peck) H.E. Bigelow of Recording and Registration (Please circle the applicable items for the characteristics.)												
Character	Characteristic values of present variety (comparison									Remarks (Measured	Characteristic values of similar varieties	
	with standard varieties									values,	(Hokuto	
	01	02	03	04	05	06	07	08	09	etc.)	(marmo22go)	18gokin)
Physio- logical property												
Dual culture												
Zone line	none								ob- served	—	09	09
formation	none								ob- served	—	01	01
Dislike- touch reaction												
Density of cultured hyphae	low	medium								03	02	02
Develop- ment of aerial hypha	low	medium								03	03	02
Color of surface of fungal flora	white		pale yellow						other	02	01	01
Color of back of fungal flora	white		pale yellow						other	02	02	02
Accommo- dativeness for temperature												
Optimal temperature for hyphal growth					24~ 26° C.		28~ 30° C.			03 21.9° C.	05 24.1° C.	04 22.5° C.
Hyphal growth rate			low				high			05 0.52 mm	06 0.70 mm	05 0.56 mm
5° C./mm Hyphal growth rate												
10° C./mm			low		medium					04 0.86 mm	05 1.16 mm	05 1.24 mm
15° C./mm			low				high			05 1.65 mm	05 1.95 mm	05 1.80 mm
20° C./mm			low		medium		high			04 2.47 mm	05 2.79 mm	05 2.60 mm
25° C./mm			low		medium		high			04 2.01 mm	05 3.29 mm	04 2.47 mm
30° C./mm			low		medium		high			04 0.91 mm	07 2.17 mm	05 1.23 mm
Morpho- logical property												
Cap												
Cross sectional shape	convex		flat	con- cave					other	02	02	02

Fungus characteristics Table of <i>Hypsizygus marmoreus</i> (Peck) H.E. Bigelow of Recording and Registration (Please circle the applicable items for the characteristics.)													
Character	Characteristic values of present variety (comparison									Remarks (Measured	Characteristic values of similar varieties		
	with standard varieties										values, (Hokuto	(marmo22go)	18gokin)
	01	02	03	04	05	06	07	08	09	etc.)			
Mottle of the surface	few	med-ium	many								02	02	02
Size of mottle	small	medium	large								03	01	03
Distribution of mottle	center part	whole part									01	02	02
Clarity of mottle	unclarity	moderate	clar-ity								03	03	03
Size			small		medium		large				03 21.2 mm	03 23.2 mm	02 20.4 mm
Color of central area	white	grey-yellow	light grey-brown	yellow-brown	grey-brown	dark tan	dark grey tan	dark grey brown	other		05 199A	05 199C	05 199B
Color of peripheral area	white	grey-yellow	light grey-brown	yellow-brown	grey-brown	dark tan	dark grey tan	dark grey brown	other		05 199C	05 199D	05 199D
Thickness			thin		medium		thick				08 9.8 mm	09 11.3 mm	0.7 8.1 mm
Fleshy			soft		med-ium		hard				05	05	07
Gill													
Color	white	cream	light orange yellow	grey-yellow					other		02 158C	02 158C	02 158C
Alignment	normal	wavy							other		02	02	02
Width	narrow	med-ium	wide								02	02	02
Density	low	Med-ium	high								02	02	02
Stipe													
Shape	long and thin	short and thin	long and thick	short and thick	Med-ium						05	05	01
Length			short		Med-ium		long				05 56.0 mm	05 53.9 mm	04 52.8 mm
Thickness			thin		medium		thick				09 17.7 mm	08 16.5 mm	05 9.4 mm
Color	white	yellow-white	grey	grey-brown					other		01 155B	01 155B	01 155B
Hair	ab-sent	few	medium	many							01	01	01
Fleshy	soft	medium	hard								03	02	02

TABLE 2-continued

Fungus characteristics Table of <i>Hypsizygus marmoreus</i> (Peck) H.E. Bigelow of Recording and Registration (Please circle the applicable items for the characteristics.)												
Character	Characteristic values of present variety (comparison									Remarks (Measured values, etc.)	Characteristic values of similar varieties	
	with standard varieties										(Hokuto	
	01	02	03	04	05	06	07	08	09		(marmo22go)	18gokin)
Ratio of maximum diameter of stem to diameter just below cap Cultural property			small		medium		large			07 2.24	06 1.91	04 1.52
Develop-ment of fruit body												
Develop-ment	group	scattered	stock							03	03	03
Optimal culture period			short		Med-ium		long			05 80 days	05 80 days	06 90 days
Length of time from fruit body-formation promotion to fruit body harvesting			short		Med-ium		long			05 23.3 days	04 21.9 days	05 22.3 days
Optimal temperature for primordial development			low		medium		high			07 19° C.	07 19° C.	05 15° C.
Optimal temperature for fruit body growth			low		Med-ium		high			05 15° C.	05 15° C.	05 15° C.
Adaptivity for illuminance												
Adaptivity for culture												
Yield												
Yield of fruit body			few		Med-ium		many			05 125.4 g	06 137.0 g	02 98.4 g
The number of productive stems												
Other property												
Bitter ingredient	none	weak	strong									
Disease resistance												
Ingredients												

Notes:
Circle shows the characteristics of 'HKHM25'.

The R.H.S. Colour Chart of The Royal Horticultural Society of London (R.H.S.), fifth edition published in 2007, The R.H.S. contact: R.H.S. Membership Department The Royal Horticultural Society PO Box 313 London, SW1P 2PE

What is claimed is:

1. A new, distinct variety of Bunashimeji Mushroom as substantially illustrated and described in the specification.

* * * * *

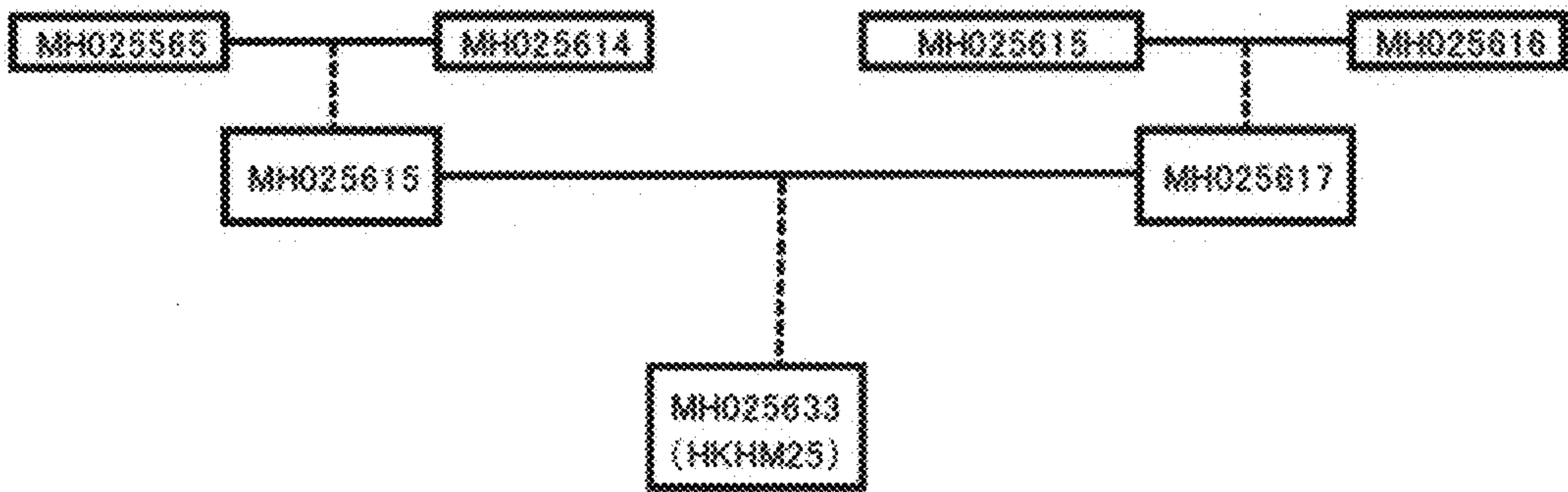


FIG. 1

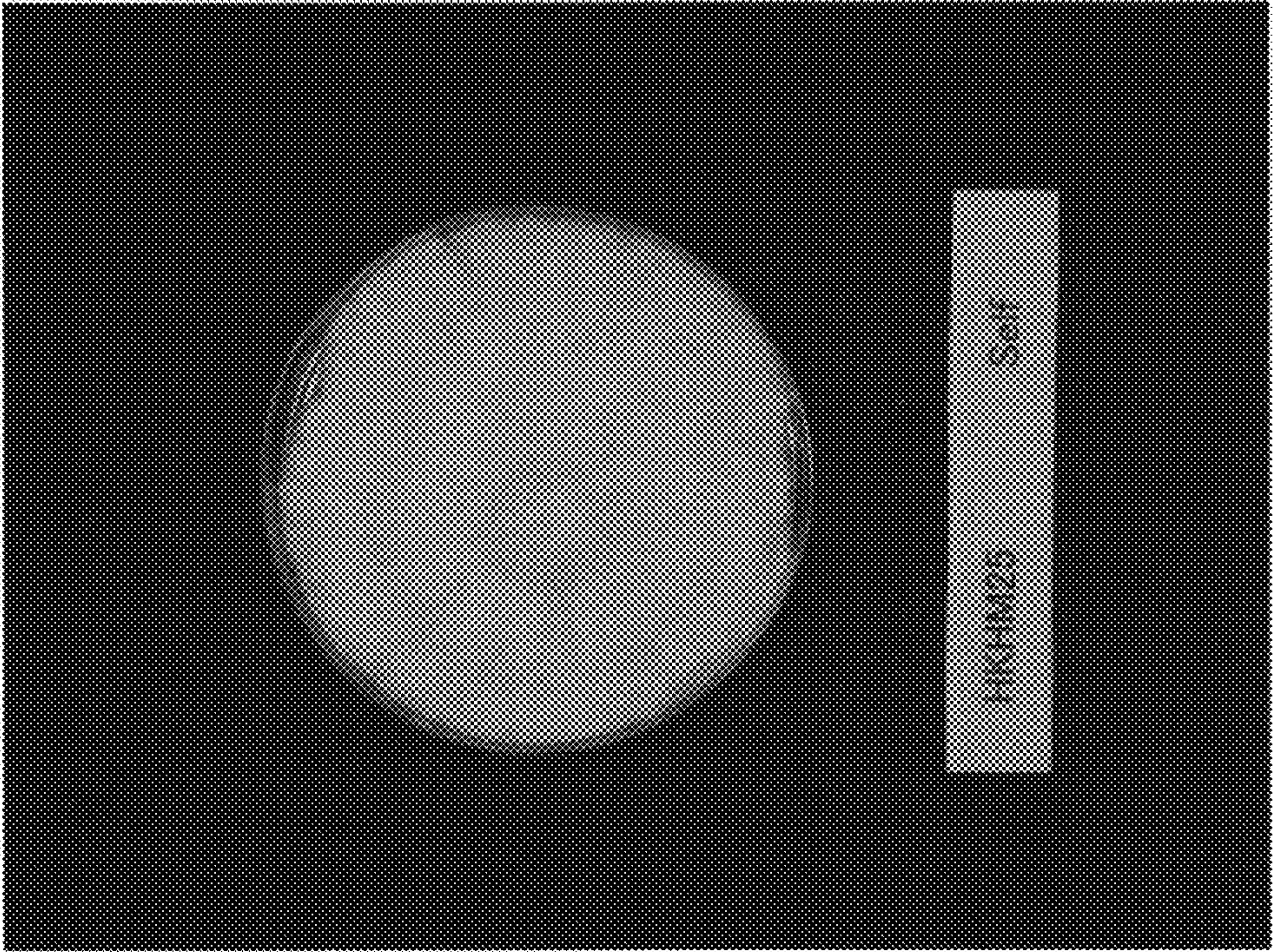


FIG. 2A

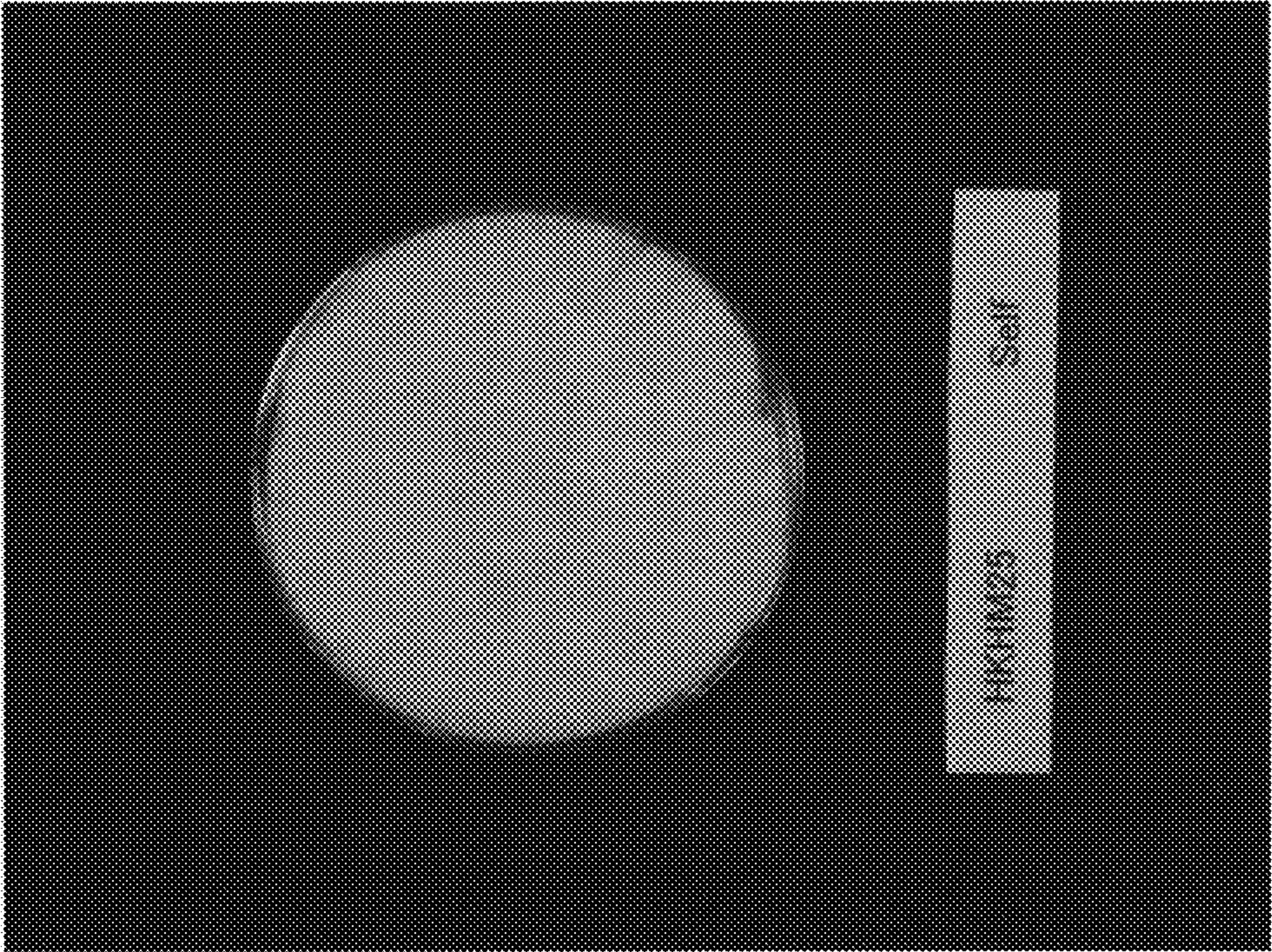


FIG. 2B

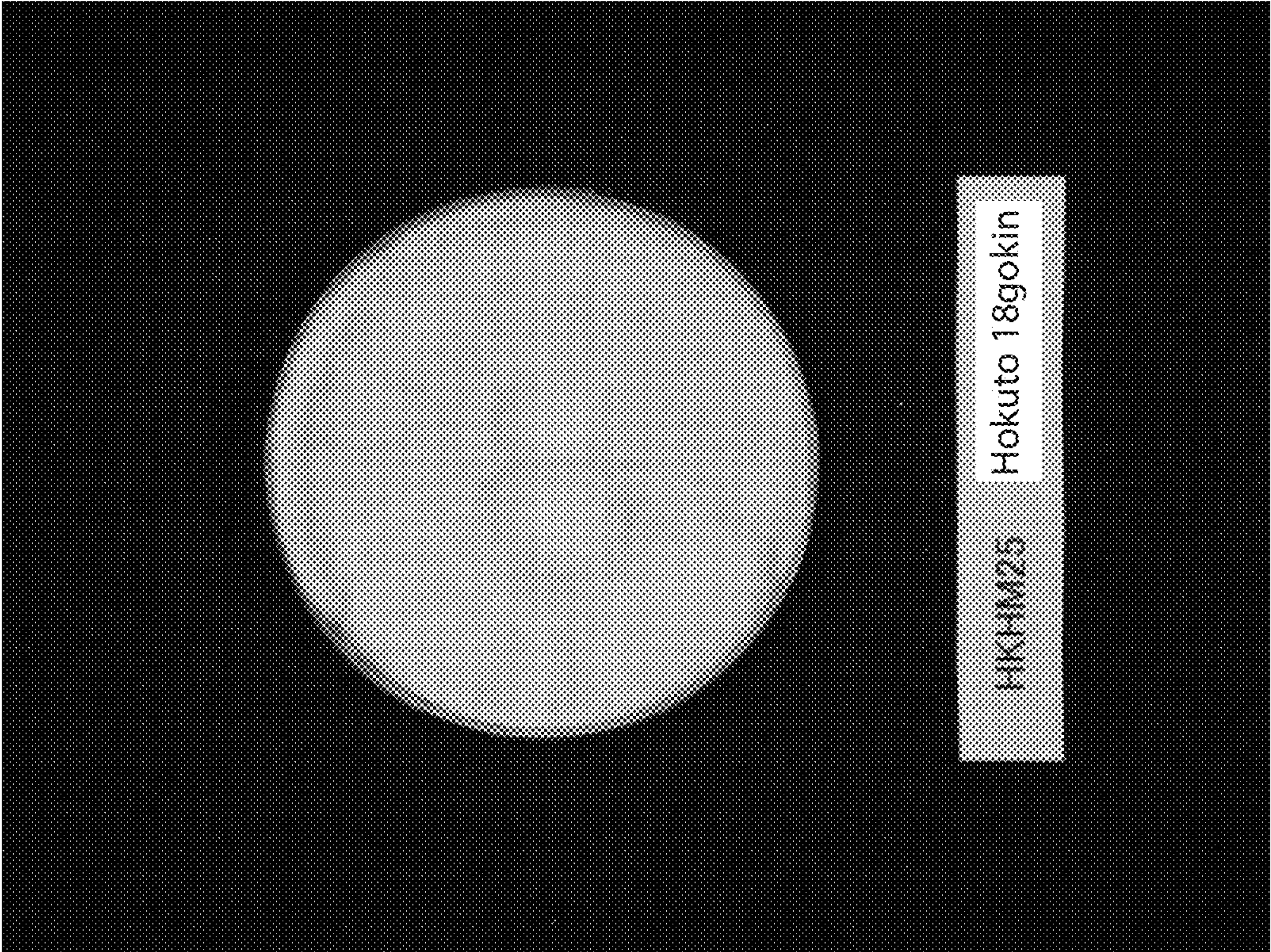


FIG. 3A

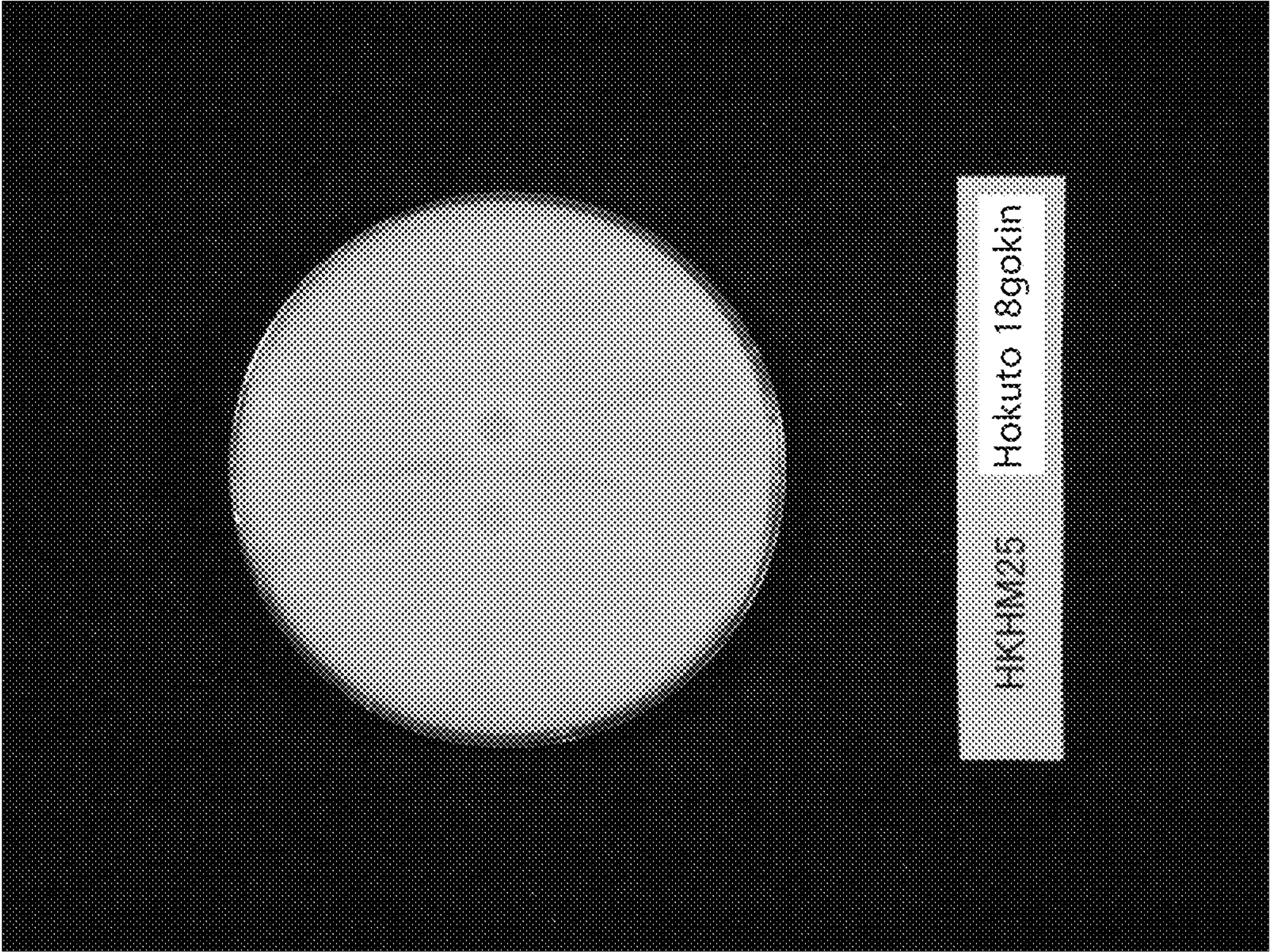


FIG. 3B

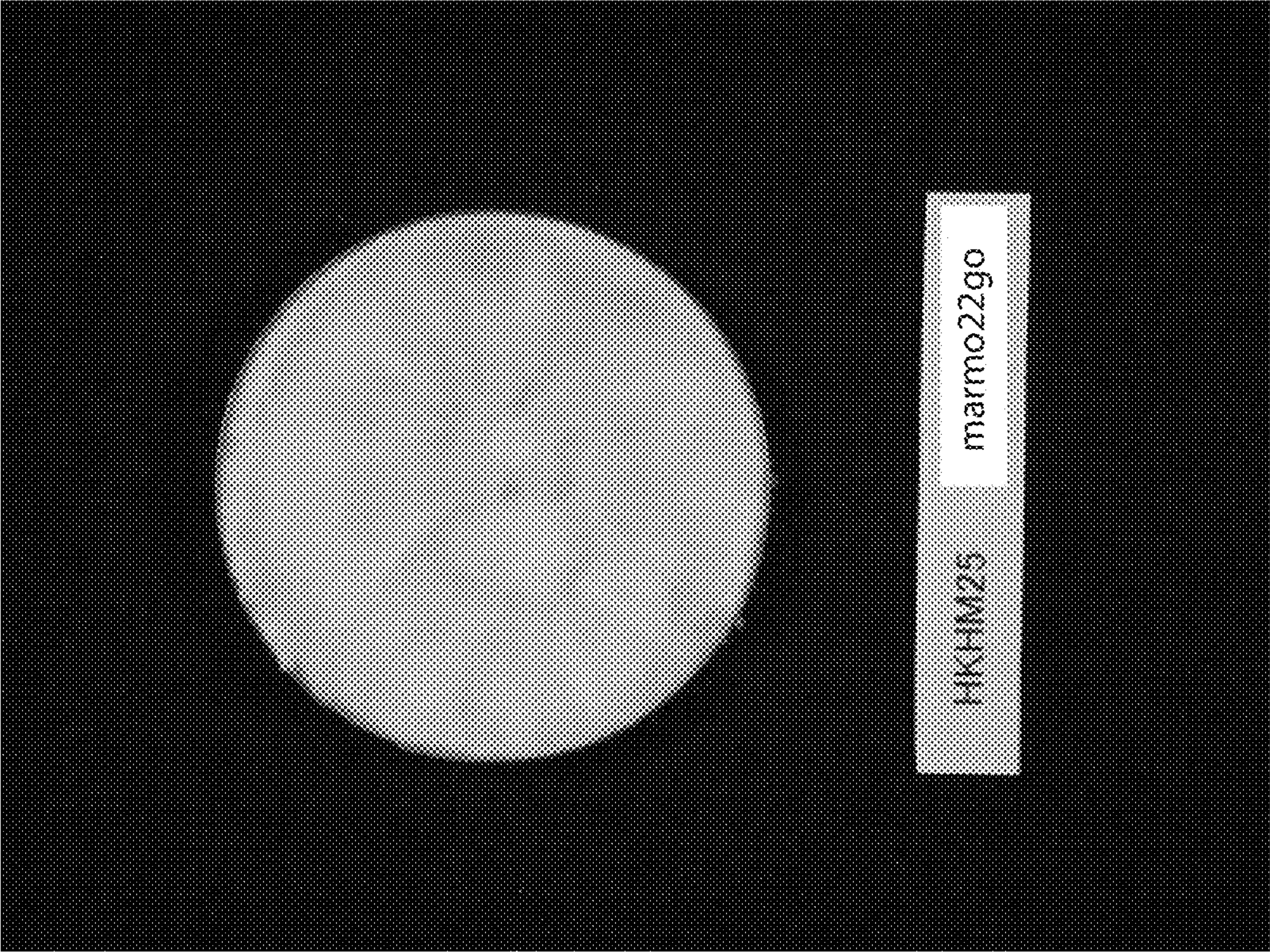


FIG. 4A

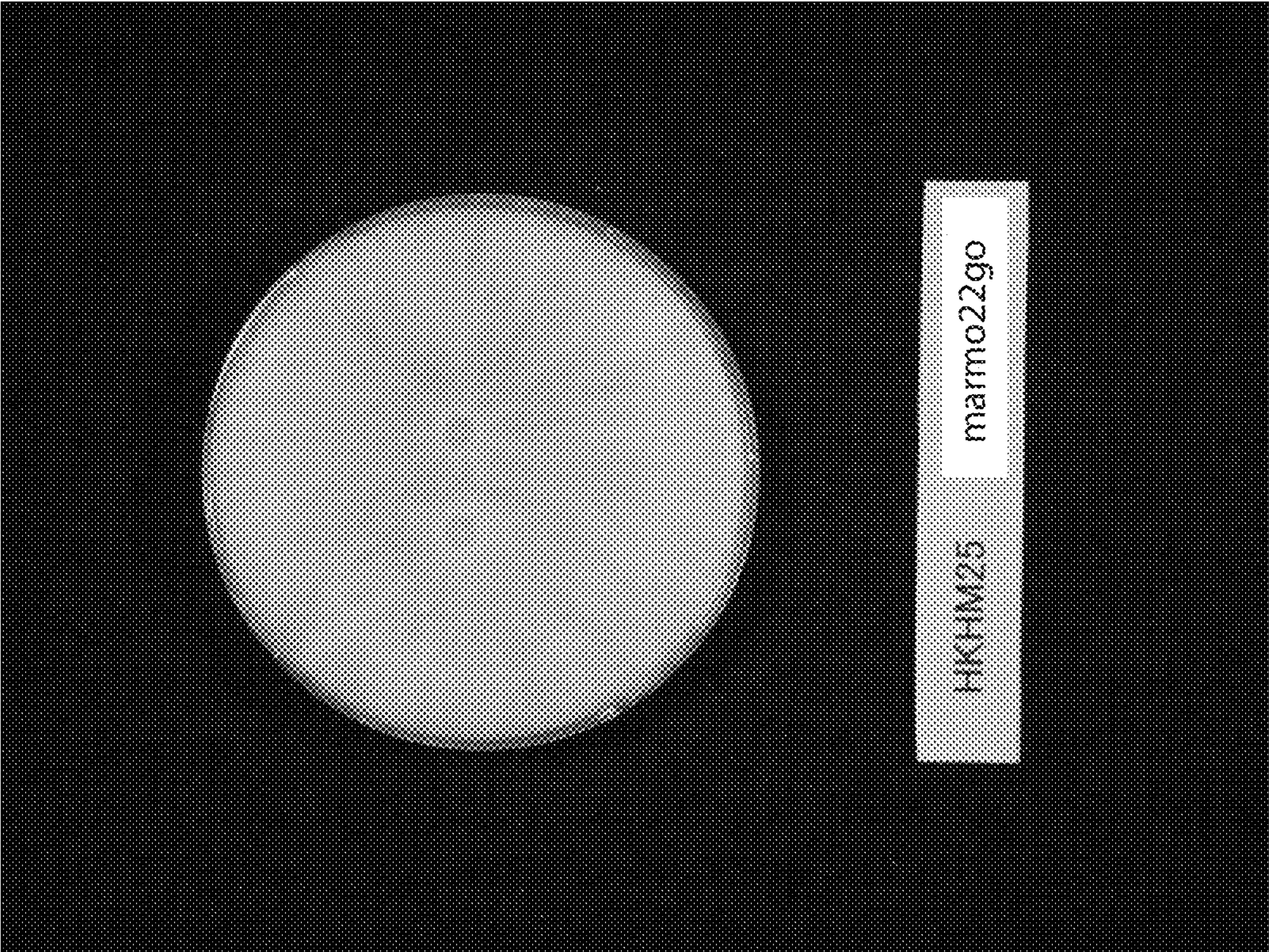


FIG. 4B

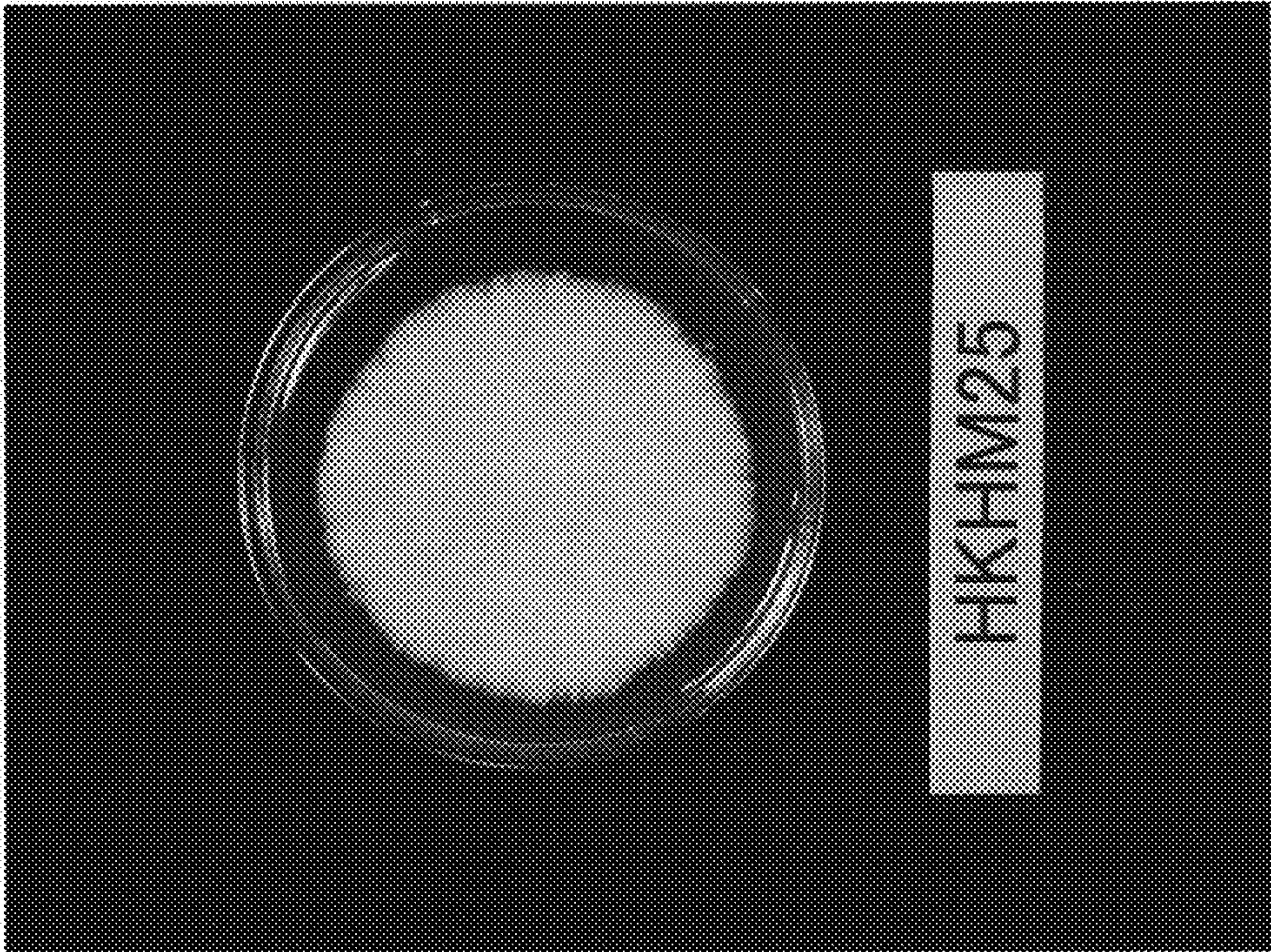


FIG. 5A

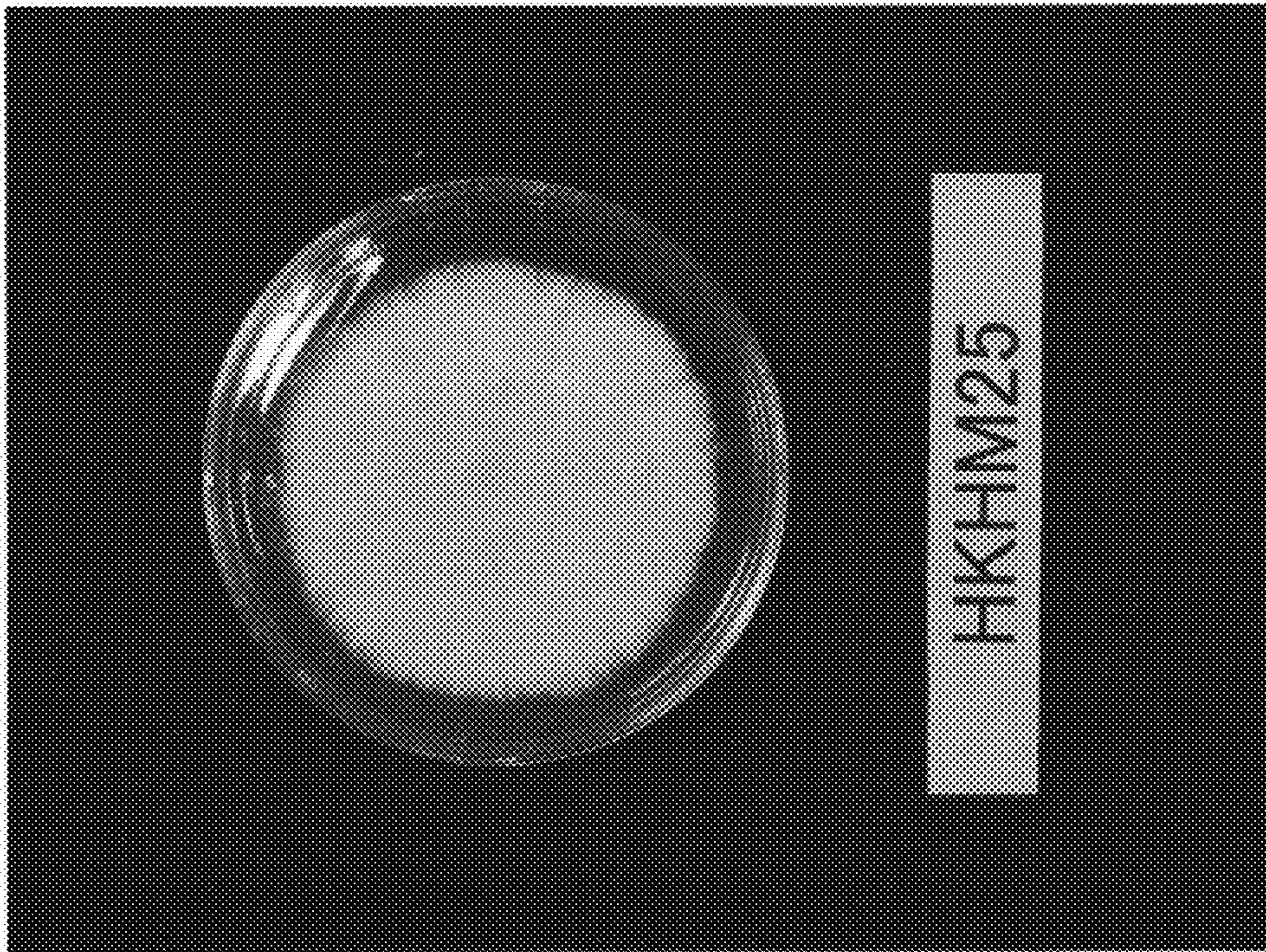


FIG. 5B

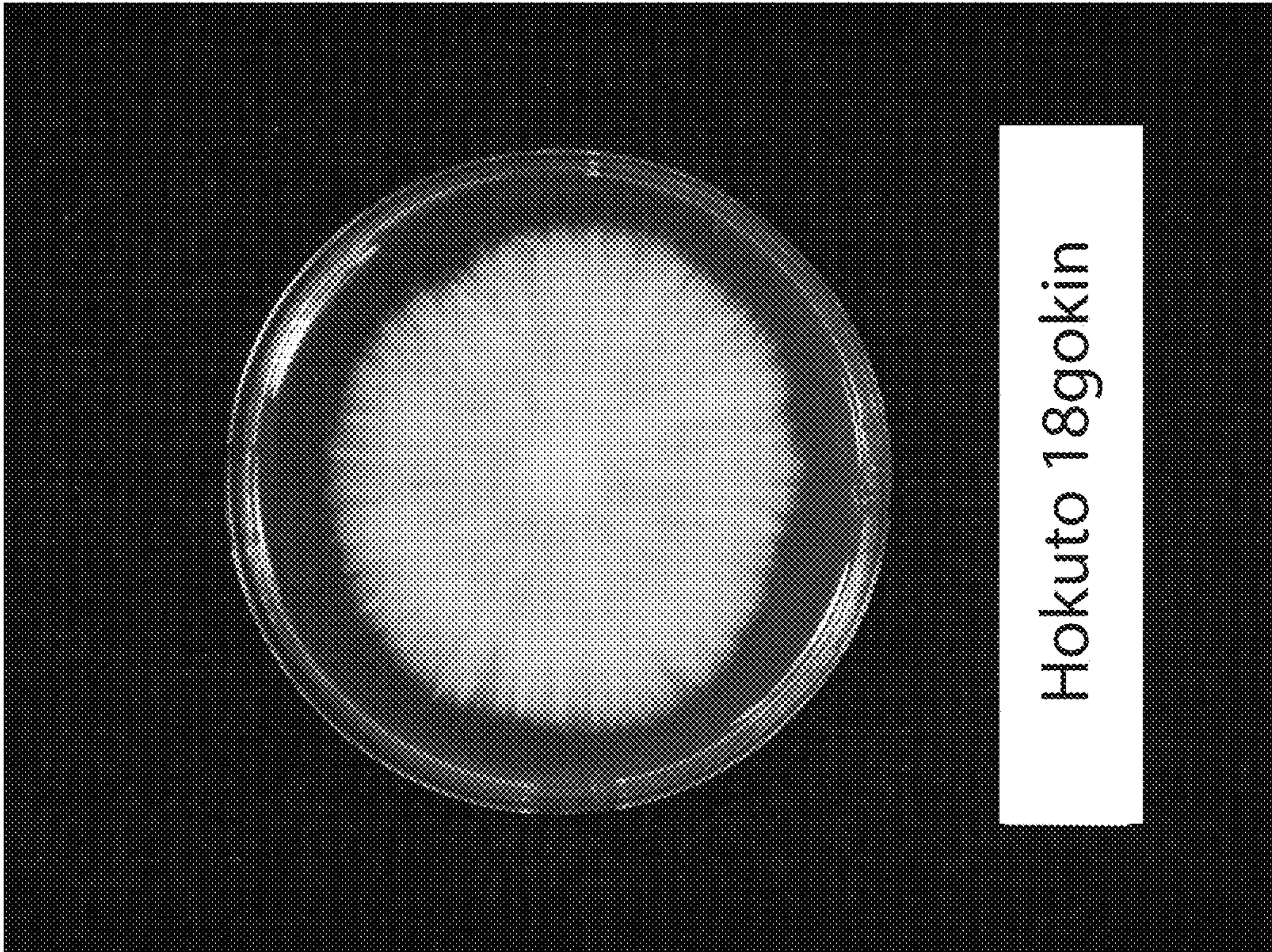


FIG. 6A

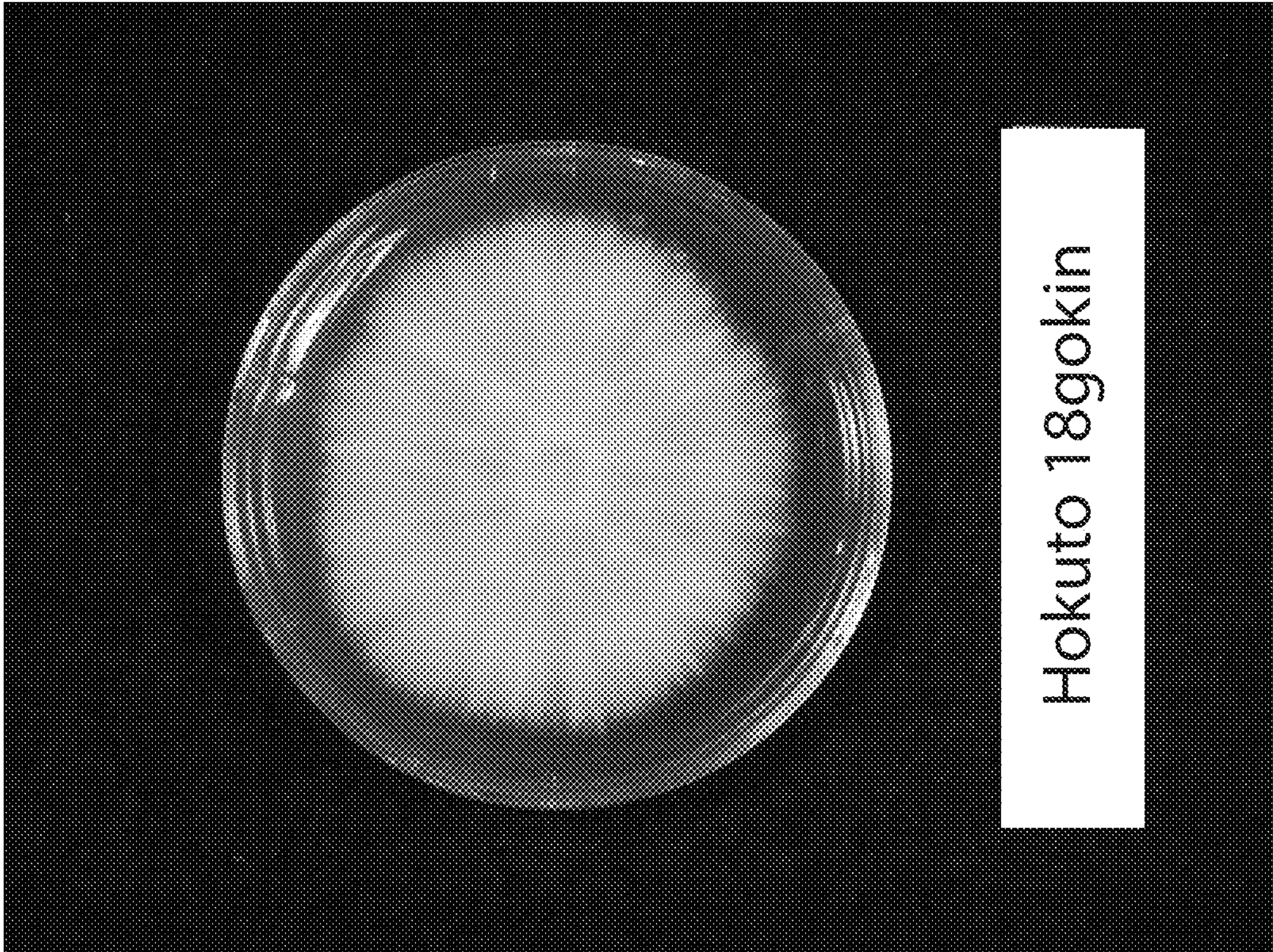


FIG. 6B

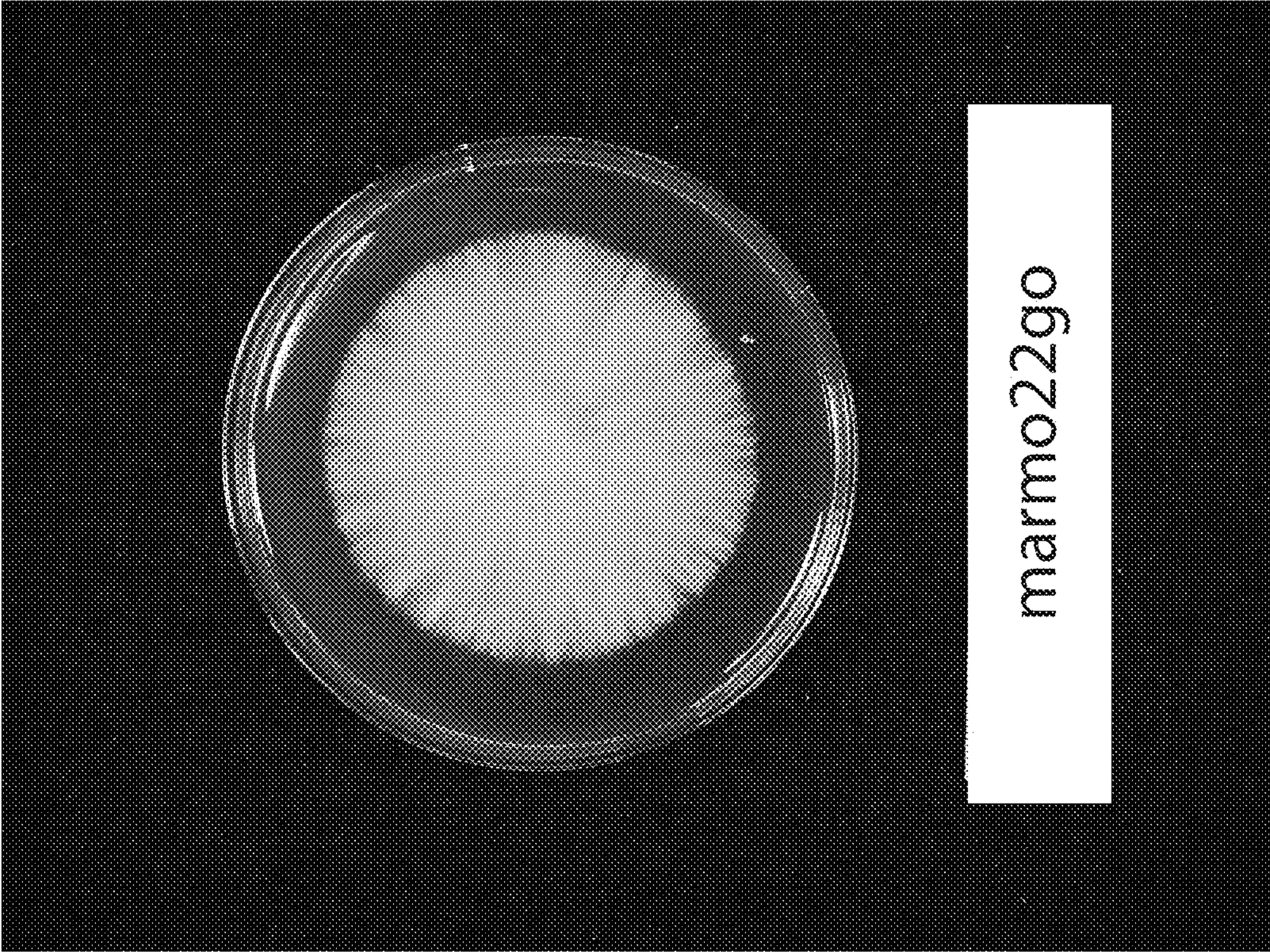


FIG. 7A

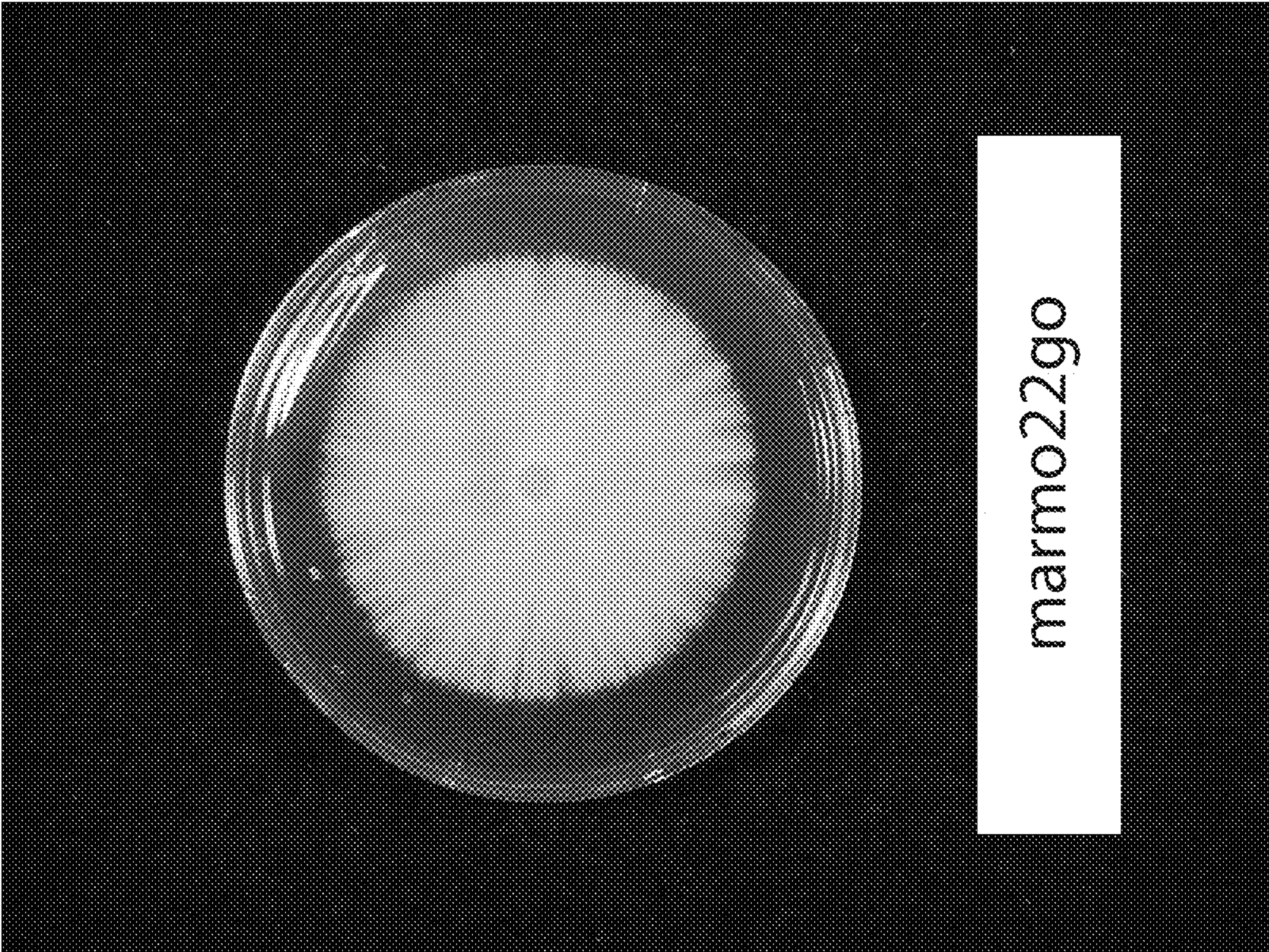


FIG. 7B



FIG. 8

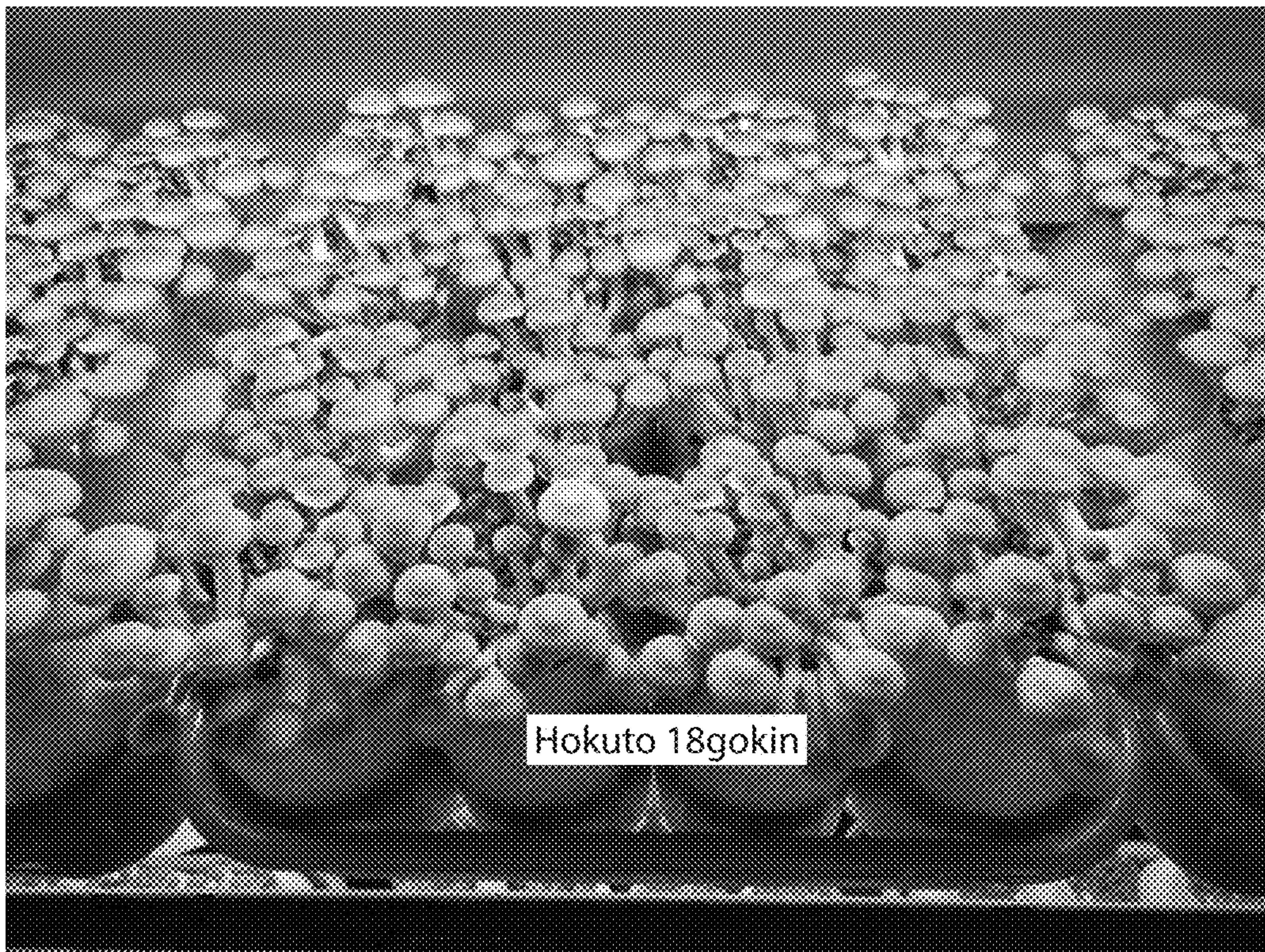


FIG. 9

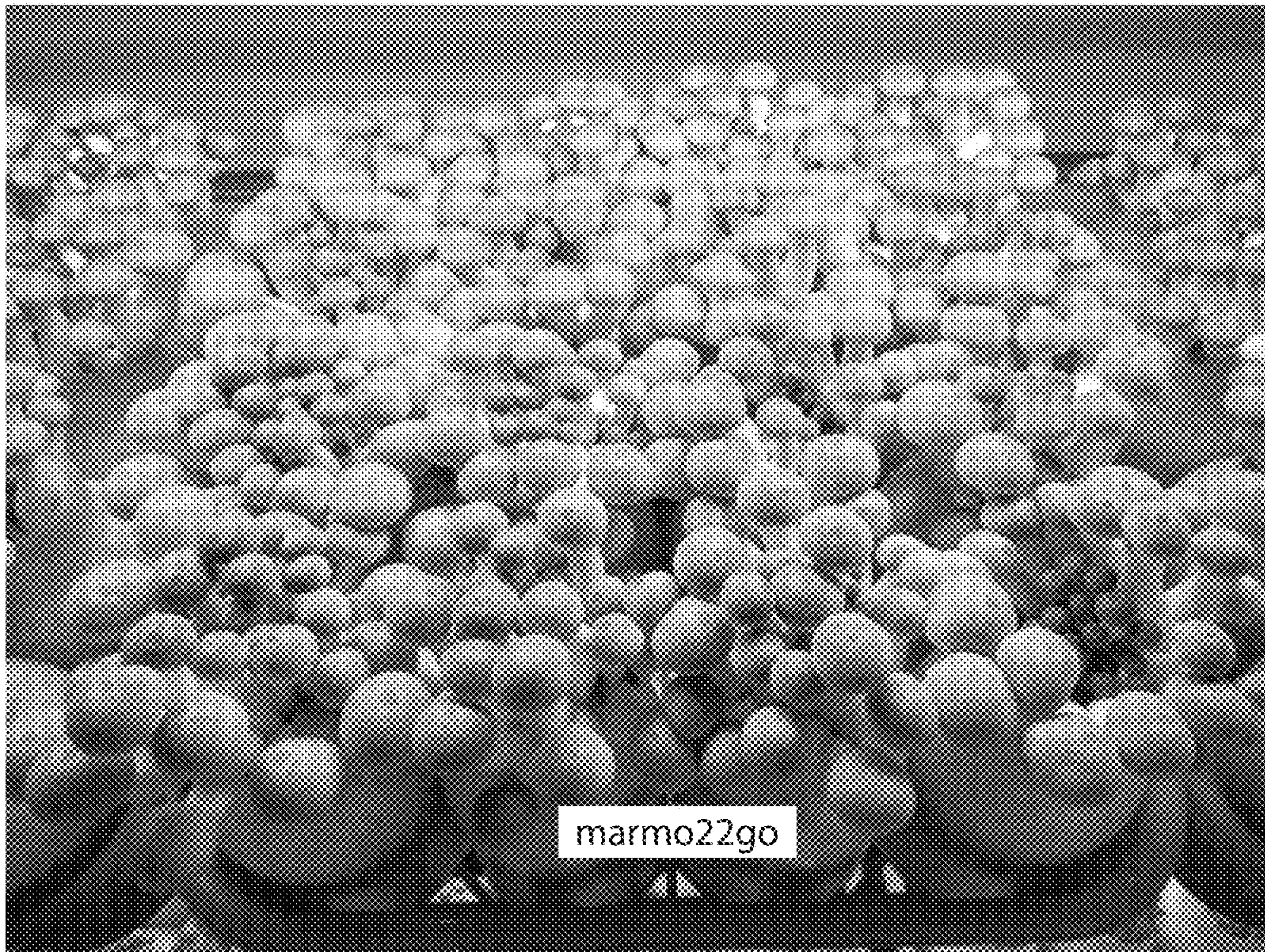


FIG. 10

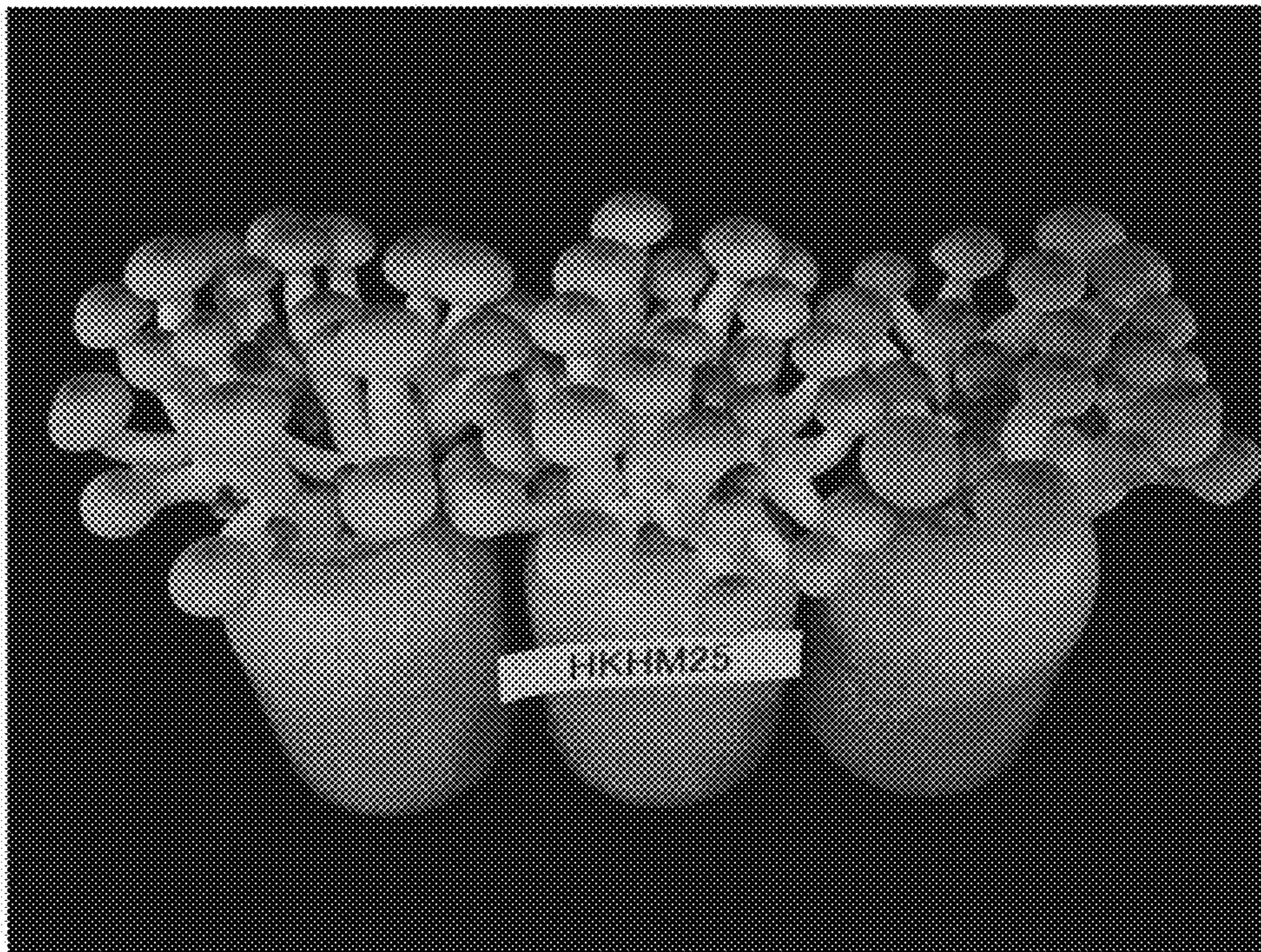


FIG. 11

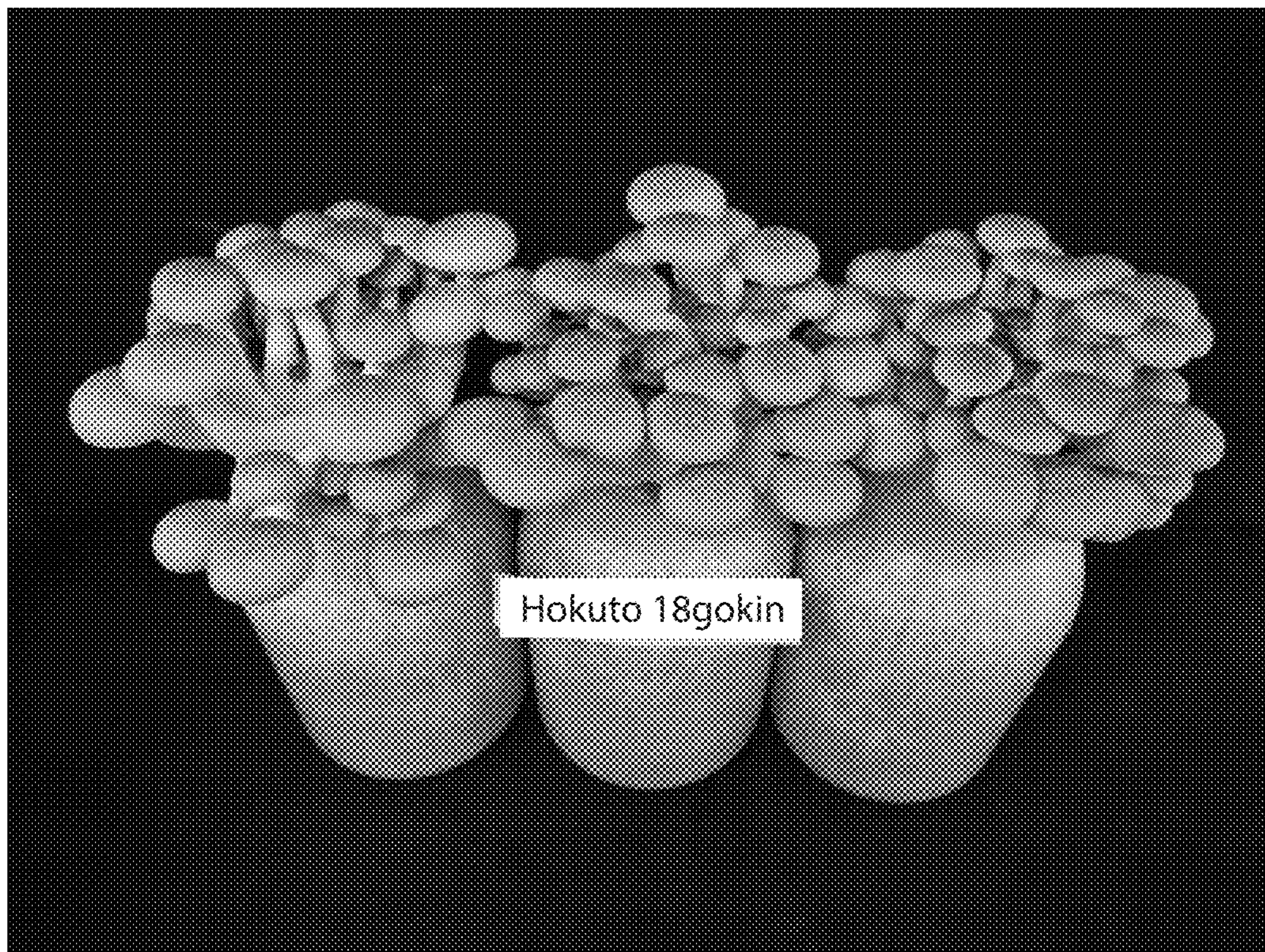


FIG. 12

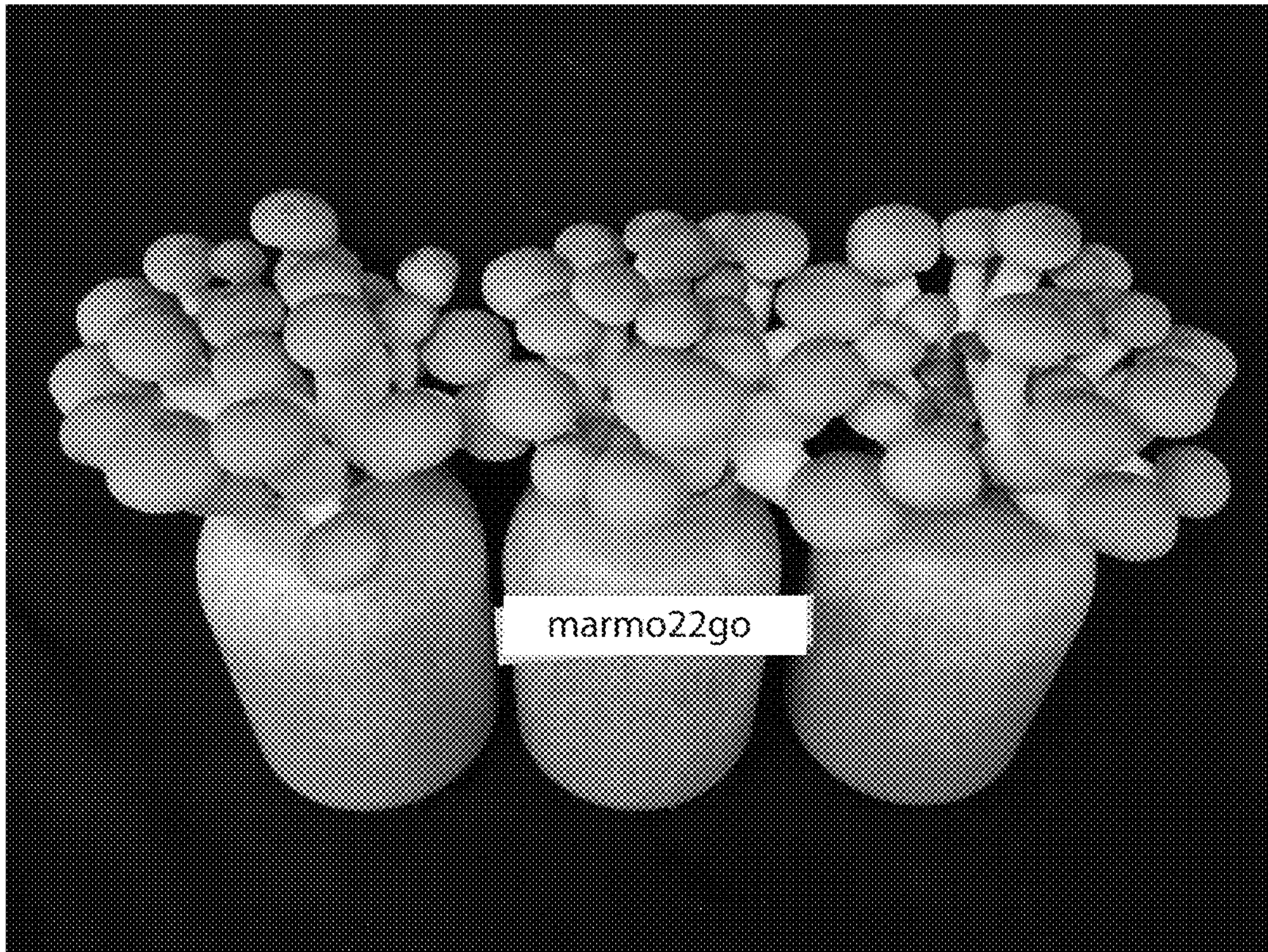


FIG. 13

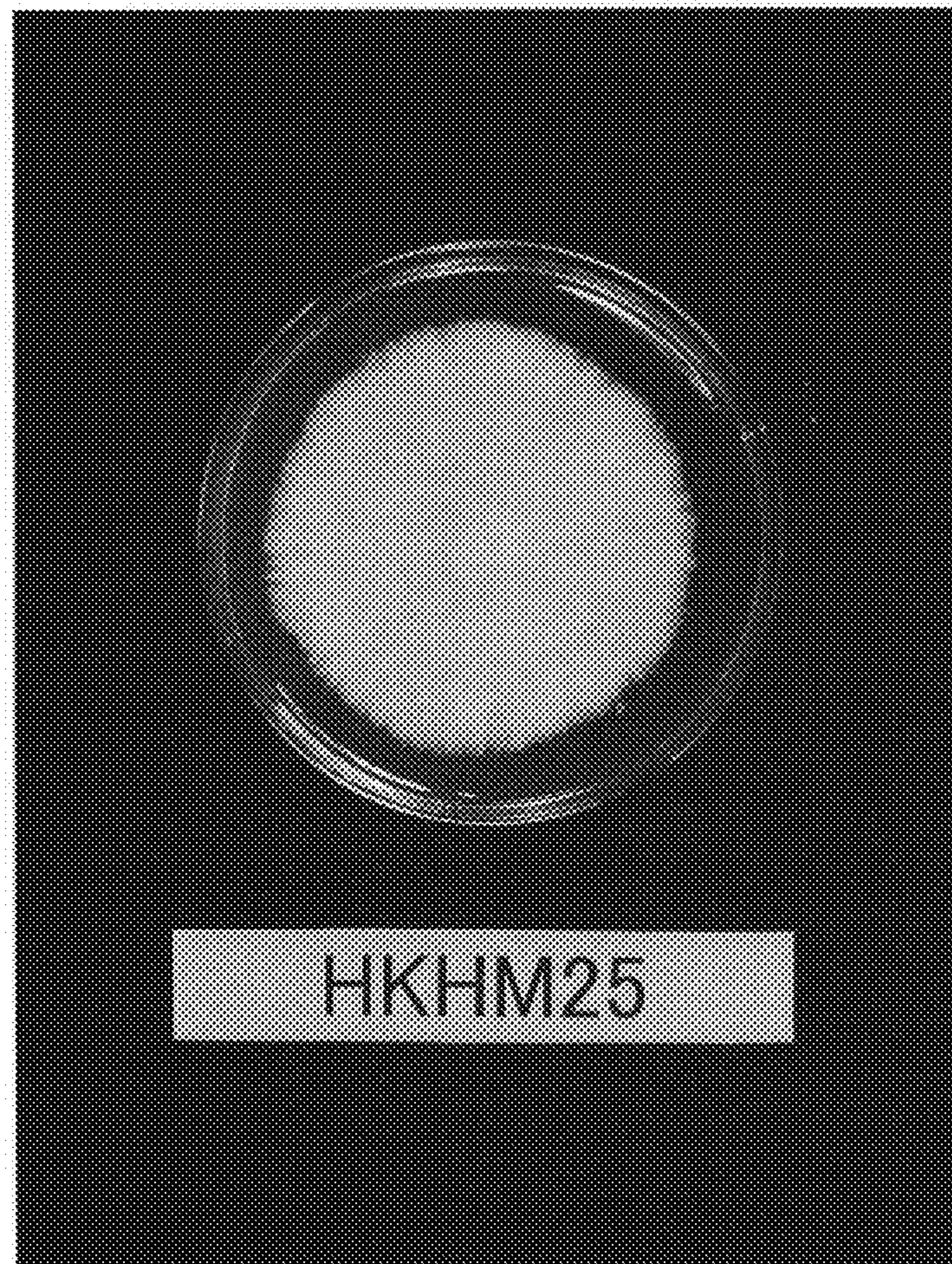


FIG. 14

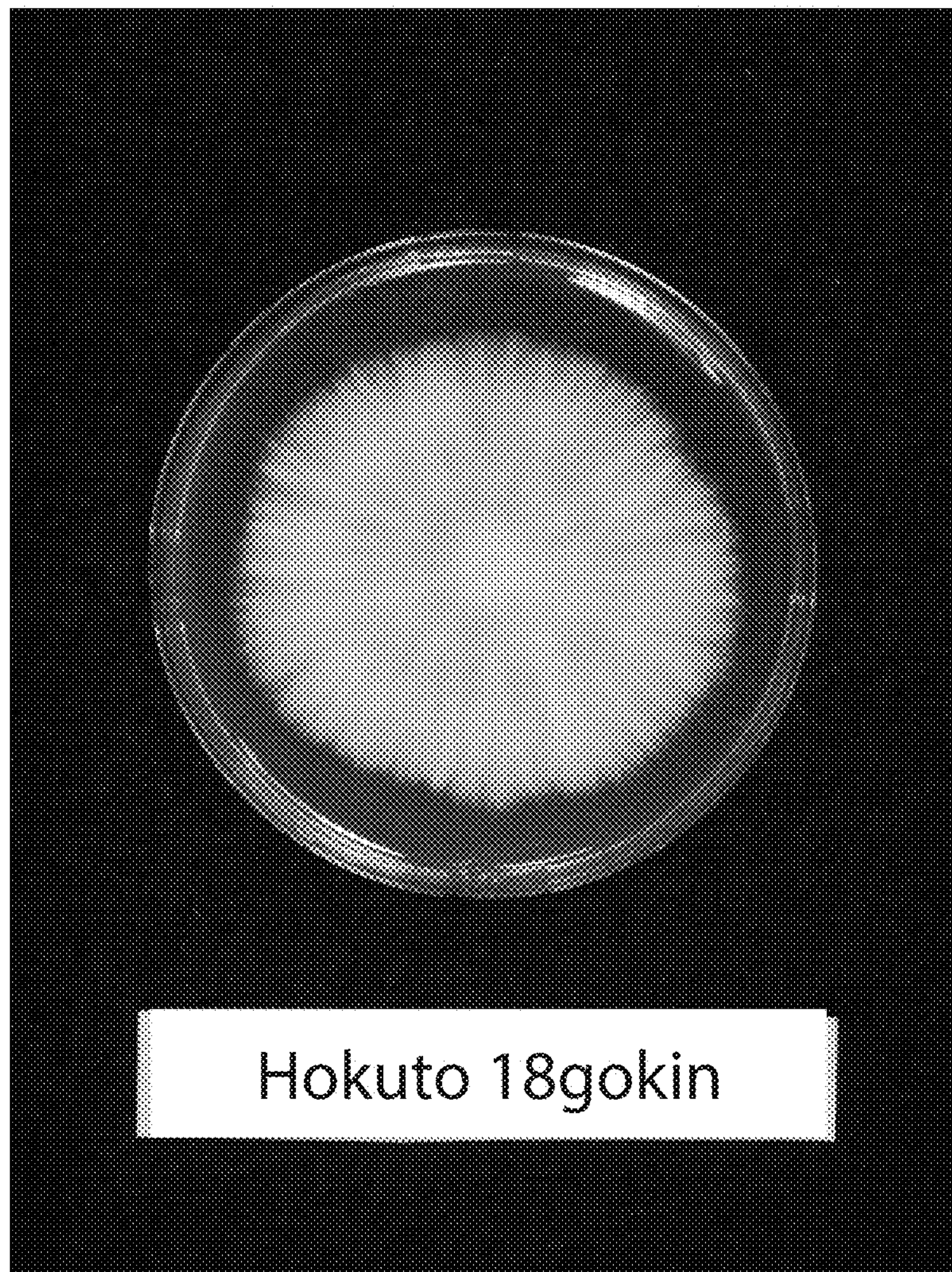


FIG. 15

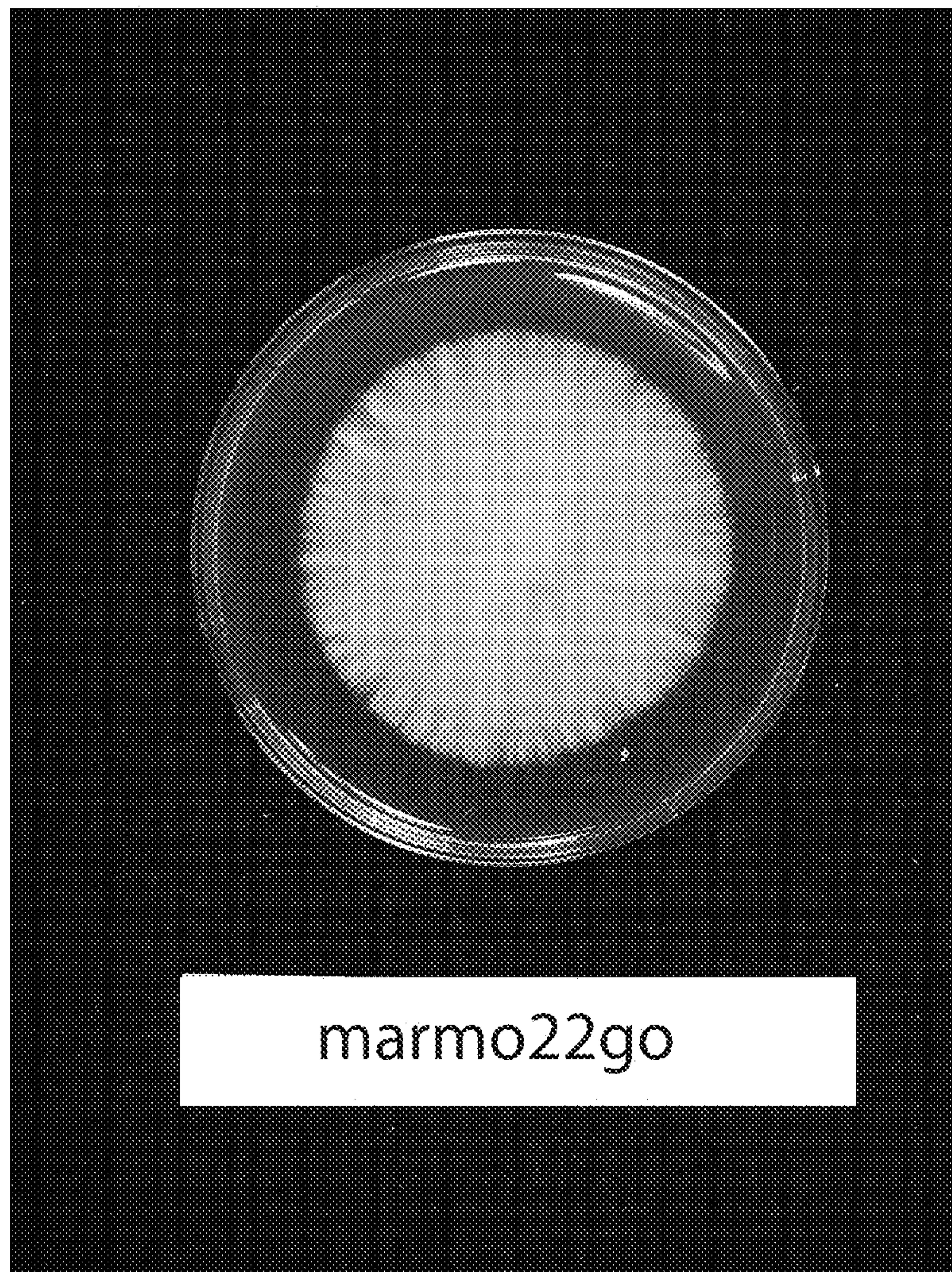


FIG. 16

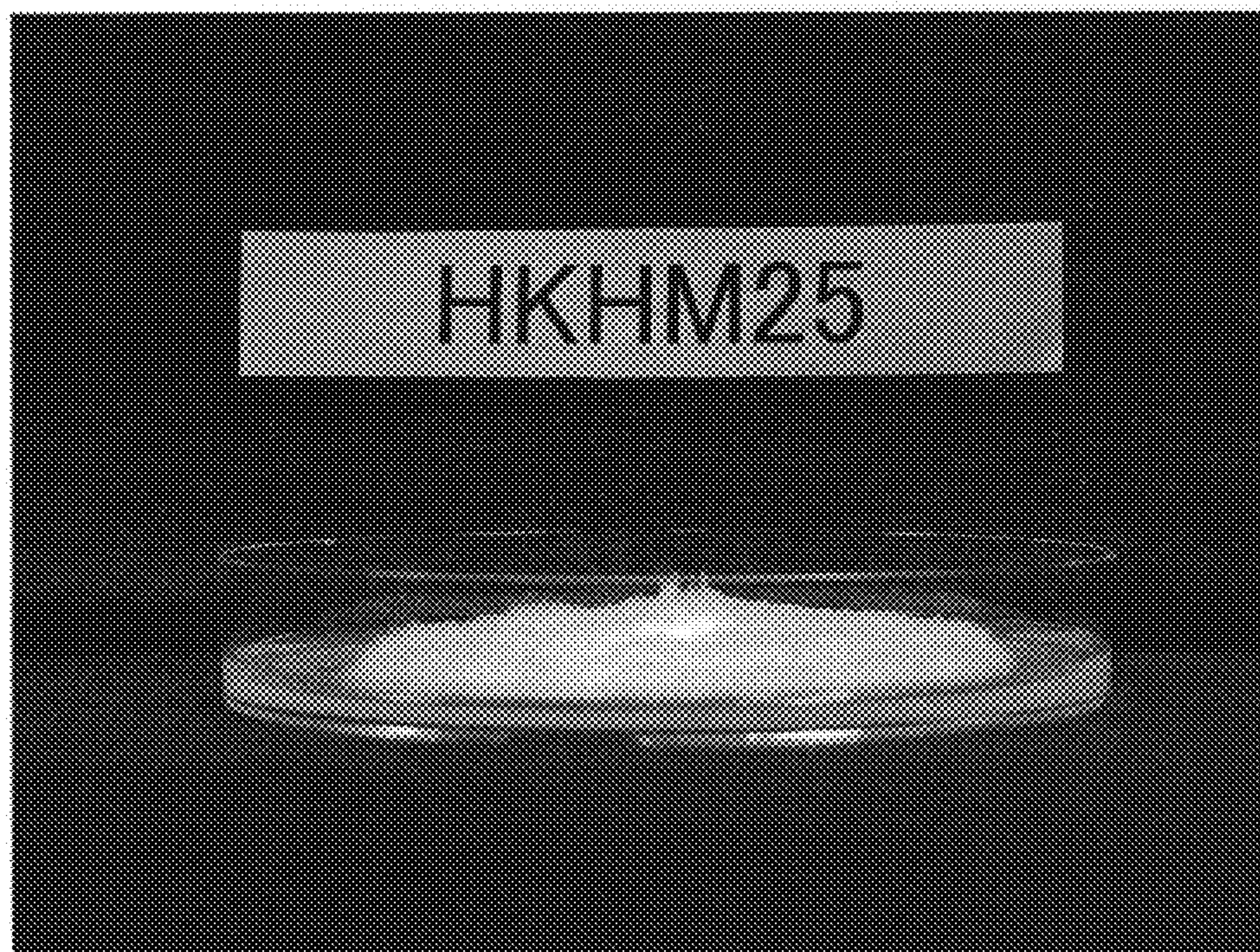


FIG. 17

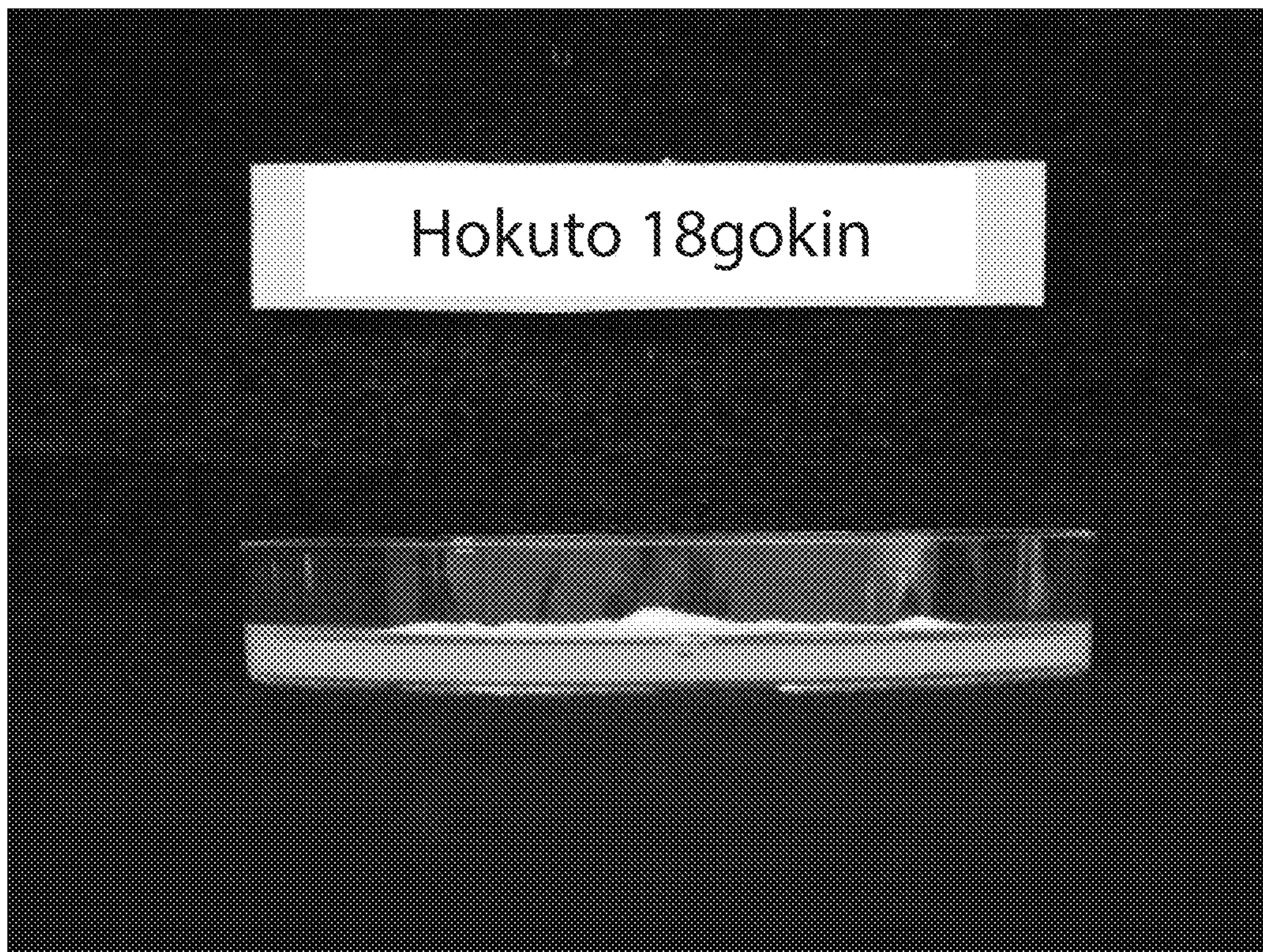


FIG. 18

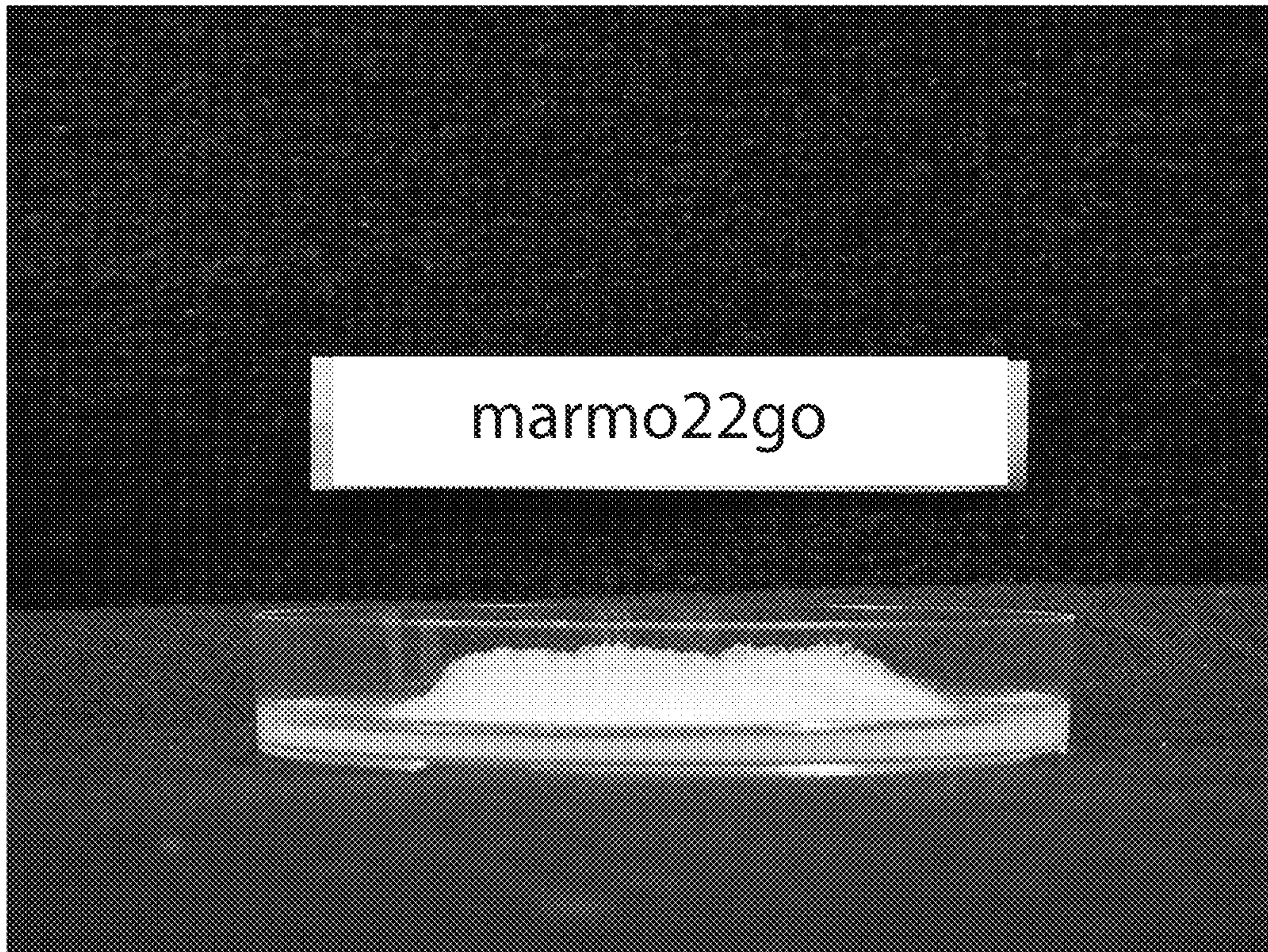


FIG. 19

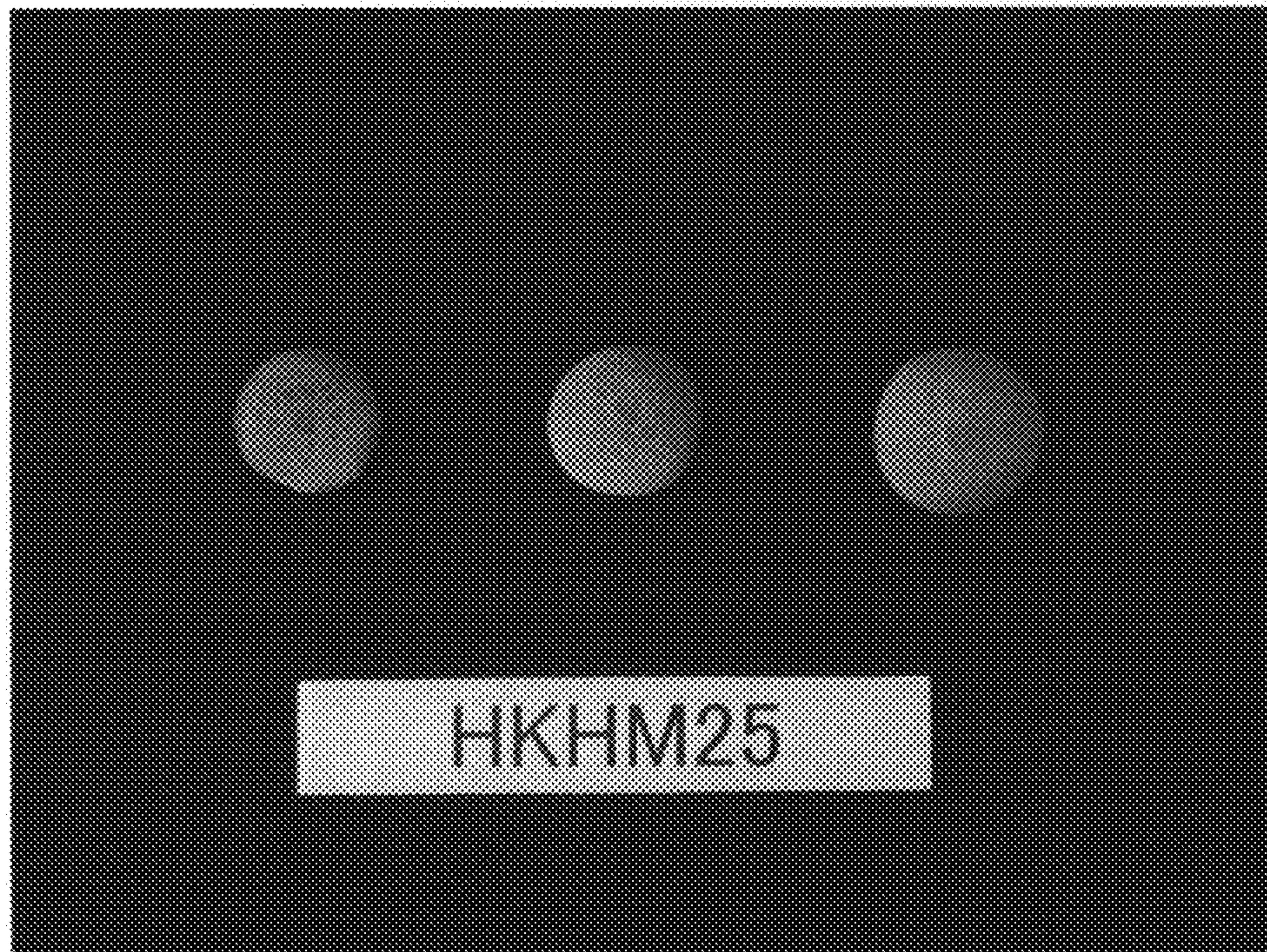


FIG. 20

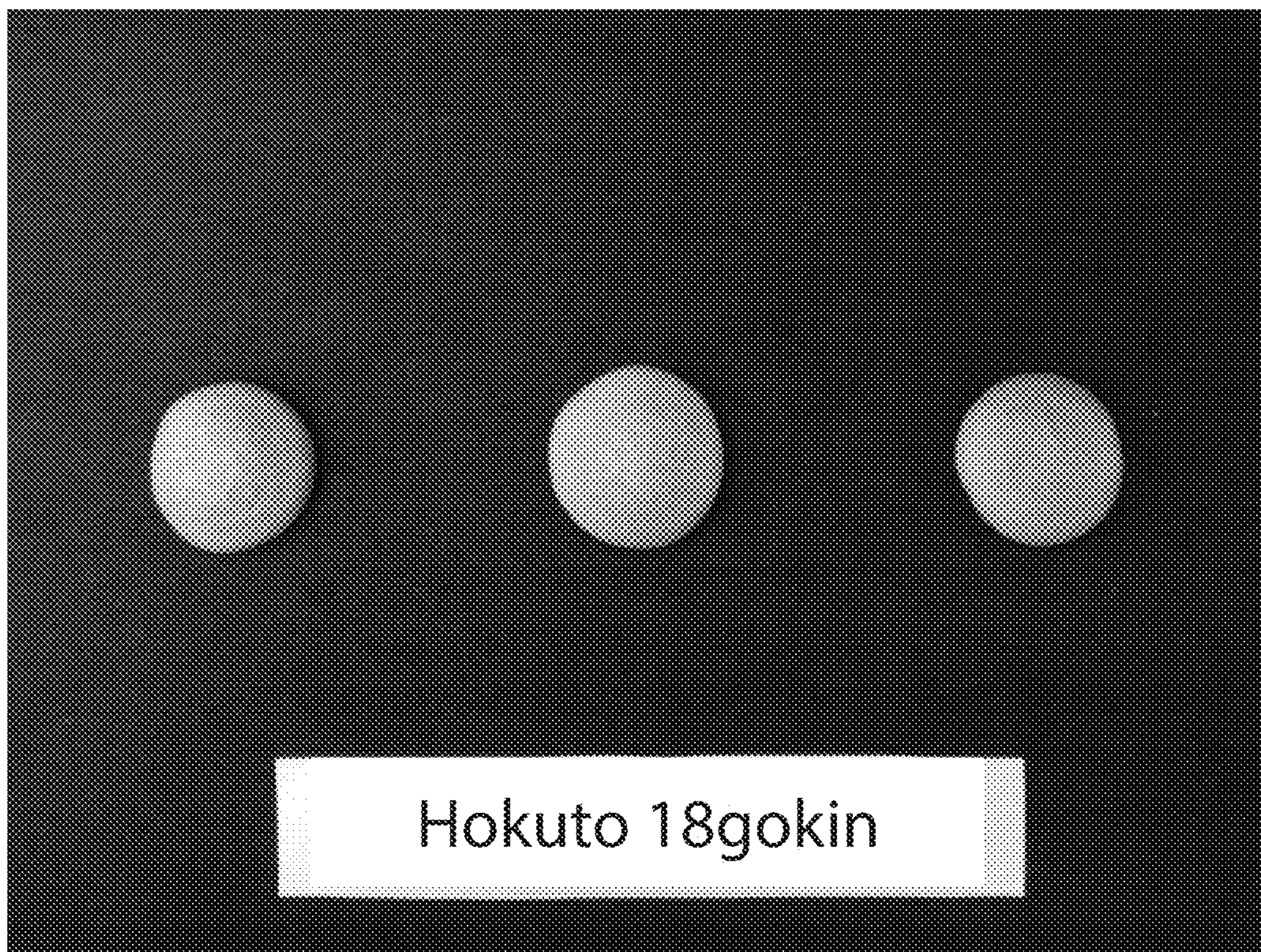


FIG. 21

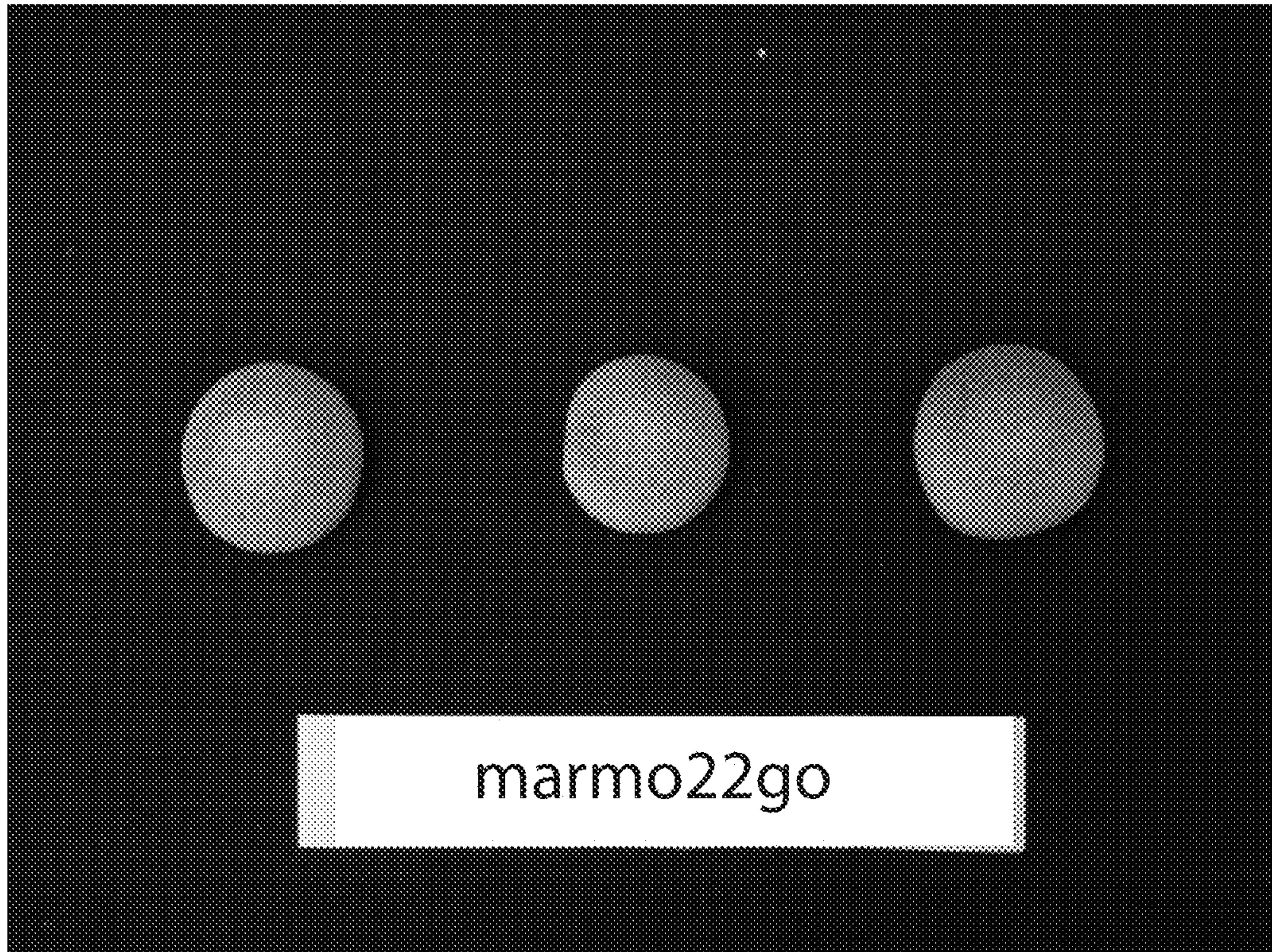


FIG. 22



FIG. 23



FIG. 24



FIG. 25

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : PP35,511 P3
APPLICATION NO. : 17/803159
DATED : November 28, 2023
INVENTOR(S) : Ayumi Nakagoshi et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page

Add Foreign Application Priority Data, it should read:
(30) Foreign Application Priority Data
March 18, 2021 (JP) 35308

Signed and Sealed this
Twentieth Day of August, 2024

Katherine Kelly Vidal
Director of the United States Patent and Trademark Office